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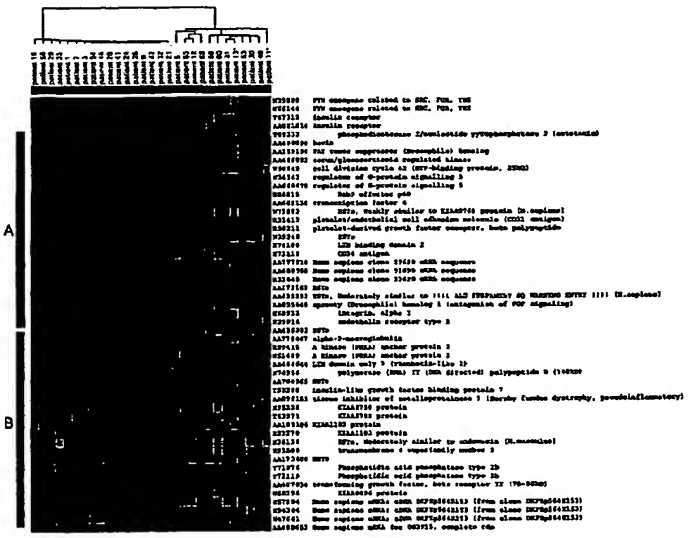
(54) Title: MICROARRAY GENE EXPRESSION PROFILING IN CLEAR CELL RENAL CELL CARCINOMA: PROGNOSIS AND DRUG TARGET IDENTIFICATION



(57) Abstract: A nucleic acid probe or a novel set of such probes in a microarray is provided. The probe or probe set is useful in the prognosis of patients with clear cell renal cell carcinoma (CC-RCC), wherein aggressive and non-agressive CC-RCC tumor types are characterized by differential expression profiles of genes that hybridize with one or more of these probes. Microarrays and kits for carrying out expression profiling of tumor and normal tissue and methods of using them are disclosed.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Microarray Gene Expression Profiling in Clear Cell Renal Cell Carcinoma: Prognosis and Drug Target Identification

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention in the field of molecular biology and medicine relates to gene expression profiling of certain types of cancer and use of the profiles for prognosis. Specifically, the differential expression of a limited set of genes permits prognosis of an aggressive form of clear cell renal cell carcinoma (CC-RCC). Other genes are up- or down-regulated in most cases of CC-RCC; these are used for early diagnosis and/or drug discovery.

Description of the Background Art

CC-RCC, the most common form of adult kidney cancer, is caused by neoplasia of proximal renal tubular epithelium. CC-RCC is a prime example of a clinically heterogeneous disease for which treatment options are largely ineffective for advanced stage tumors. The cancer is more common in men than women, especially men over 55 years of age. It affects approximately 3/10,000 people; 18,000 new cases arise in the U.S. annually, of which about 8,000 result in death; worldwide fatalities are estimated to exceed 100,000 in 2001. CC-RCC represents 2% of all malignancies and 2% of all cancer-related deaths. Approximately 30% of patients present with metastatic disease and life expectancies averaging only 9 months.

RCC, originally named *hypernephroma*, was found to originate in the proximal renal tubule (Oberling *et al.*, *Nature* (1986) **186**:402-403) leading to its renaming to *renal cell adenocarcinoma* or *renal cell carcinoma*. RCC has been subdivided into clear, papillary, granular, and mixed cell variants based on cytoplasmic features. But the prognosis of RCC is based on staging and histological grading rather than the above classification.

A subtype of renal neoplasia with granular cell features, *renal oncocytoma*, which had excellent prognosis is described by Klein *et al.*, *Cancer* (1976) **38**:909-914. Thoenes *et al.*, *Virchows Arch B Cell Pathol Incl Mol Pathol.* (1985) **48**:207-217, describe a subtype of RCC with clear cell features, closely resembling an experimental renal tumor in rats, naming it *chromophobe renal cell carcinoma*. Fleming *et al.*, *Histopathology* (1986);**10**:1131-1141 describe yet another

renal tumor, originating from the collecting ducts, named *collecting duct carcinoma*. Overlap of granular and clear cell features among tumors with marked clinical, pathologic, and phenotypic differences promoted the need for a new classification. Thoenes *et al.* (*Pathol Res Pract.* (1986) **181**:125-143) proposed a new classification for renal tumors of tubular epithelial origin (the "Mainz classification") based on conventional histopathologic criteria that include all the new entities described above.

The Mainz classification is now widely accepted; cytogenetic studies have confirmed characteristic genetic alterations of each tumor type (Yoshida *et al.*, *Cancer Res* (1986) **46**:2139-2147; Kovacs *et al.*, *Proc Natl Acad Sci USA* (1988) **85**:1571-1575 and *Histopathology* (1993) **22**:1-8; Walter *et al. Cancer Genet Cytogenet.* (1989); **43**:15-34).

The term RCC embraces a group of renal cancers all of which are derived from the renal tubular epithelium but each with distinct clinical, pathologic, phenotypic, and genotypic features.

Tumor Type	Relative Frequency
Renal Cell Carcinoma:	
Clear Cell	70%
Chromophil (eosinophil, basophil)	15%
Chromophobe (typical, eosinophil)	5%
Collecting Duct Carcinoma	2%
Renal Oncocytoma	5%

CC-RCC is the most common adult renal neoplasm (70%). The tumor can be 1 cm in diameter when discovered (usually incidentally), or as bulky as several kilograms. Most often it manifests with pain, as a palpable mass or with hematuria; a variety of paraneoplastic syndromes have been described. CC-RCC may first manifest with metastases after being clinically silent for years. The characteristic gross appearance of the tumor is solid, lobulated, and yellow, with variegation due to necrosis and hemorrhage. Tumor may be well circumscribed, or may invade the perirenal adipose tissue or the renal vein. Cystic degeneration is common, though some tumors are predominantly cystic (Hartman *et al.*, Urology (1986) 28:145-153). Of the 70% of patients with initially non-metastatic disease, approximately 30% relapse after surgery and usually succumb (Levy *et al.*, *J. Urolog. 159*:1163-1167 (1999); Ljungberg, B *et al.*, *BJU Intl. 84*: 405-411 (1999)).

The most common and consistent genetic finding in CC-RCC has been chromosomal (3p) loss (Tajara *et al.*, *Cancer Genet Cytogenet* (1988) 31:75-82), along with a mutation in the von Hippel-Lindau (VHL) gene in the other chromosome 3. In about 50% of sporadic CC-RCC cases, the VHL gene, located in 3p25, was mutated (Gnarra JR *et al.*, (1994) *Nature Genet* 7:85-90). Reports of frequent loss of heterozygosity (LOH) in chromosome 3p13 and 3p14 suggested that other CC-RCC related genes exist in this region. Indeed, there are families with familial CC-RCC not associated with the VHL gene or chromosome 3 translocations (Teh, BT *et al.*, 1997, *Lancet 349*:848-849), further supporting the notion that other CC-RCC genes exist.

To date, there have been no effective tools to identify those patients who will go on to relapse. Though the stimulus for RCC neoplastic transformation has not been identified, many associations with etiologic factors have been evaluated. Cigarette smoking is a prime risk factor. Incidence of CC-RCC is significantly increased in endstage renal patients who develop acquired cystic kidney disease. Although the tumors typically arise in the renal cortex, they may invade the renal vein and extend into the inferior vena cava. Paraneoplastic syndromes such as hypercalcemia and hepatic dysfunction in the absence of liver metastases have been reported.

The Union Internationale Contre le Cancer (UICC) recently developed an improved system for classifying CC-RCC known as the "TNM" classification (referring to tumor, lymph node and metastasis). T, N, and M categories are determined by physical examination and imaging. (Sobin, L.H. *et al.*, eds., *TNM classification of malignant tumors*. 5th ed. (John Wiley & Sons, New York 1997). This system is set forth in the table below.

Approximately one-third of initially diagnosed CC-RCC patients present with metastatic disease, and 40% of individuals undergoing surgical resection or radical nephrectomy will eventually develop metastasis. Among individuals with metastatic disease, approximately 75% exhibit lung metastasis, 36% have lymph node and/or soft tissue involvement, 20% have bone involvement, and 18% have liver involvement. The literature also reports low incidences of metastasis in contralateral adrenal glands, brain, uvula, diaphragm, and digits (Levy *et al.*, supra). Spontaneous regression of metastases after nephrectomy occurs primarily in men with pulmonary metastasis and are not equated with long-term cure. The frequency of spontaneous regression is only 0.4% and may reflect the development and/or enhancement of immune responses.

TNM Clinical Classification

T--Primary Tumor

TX Primary tumor cannot be assessed
T0 No evidence of primary tumor
T1 Tumor is ≤7.0 cm in greatest dimension, limited to the kidney
T2 Tumor is >7.0 cm in greatest dimension, limited to the kidney
T3 Tumor extends into major veins or invades adrenal or perinephric tissues but not beyond Gerota fascia
- T3a Tumor invades adrenal gland or perinephric tissues but not beyond Gerota fascia
- T3b Tumor grossly extends into renal vein(s) or vena cava below diaphragm
- T3c Tumor grossly extends into vena cava above diaphragm

T4 Tumor invades beyond Gerota fascia

N--Regional Lymph Nodes *(hilar, abdominal para-aortic, and paracaval)*

NX Regional lymph nodes cannot be assessed
N0 No regional lymph node metastasis
N1 Metastasis in a single regional lymph node
N2 Metastasis in more than one regional lymph node

M--Distant Metastasis

MX Distant metastasis cannot be assessed
M0 No distant metastasis
M1 Distant metastasis present

pTNM Pathological Classification: corresponds to the T, N, and M categories.

G--Histopathological Grading

GX Grade of differentiation cannot be assessed
G1 Well differentiated
G2 Moderately differentiated
G3, 4 Poorly differentiated/undifferentiated

Stage Grouping

	M	N	M
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T1	N1	M0
	T2	N1	M0
	T3	N0, N1	M0
Stage N	T4	N0,N1	M0
	Any T	N2	M0
	Any T	Any N	M1

Conventional treatment of primary CC-RCC is surgical excision. However, metastasis limits long term survival. In patients with symptomatically advanced CC-RCC, palliative nephrectomy and other tumor excisions may be the only therapeutic option (Ljungberg *et al.*, supra). Radiotherapy appears to have only limited palliative effects, as CC-RCC's appear to be relatively radio-resistant. Chemotherapy, usually with vinblastine, hydroxyurea and/or BCNU, also shows limited efficacy and response rates to prolonged infusion of 5-fluorouracil range from <10% to 20% decrease in tumor size. (Dutcher *et al.*, *Proc Annu Meet Am Soc Clin Oncol* (1996) *15*:A725). Hormonal therapy has also yielded disappointing results (Bukowski, *Cancer* (1997) *80*:1198-1220).

Immunotherapy with cytokines such as interferons and interleukin-2 (Proleukin® from Chiron), and combinations of these agents is considered an encouraging area of therapeutic development.

The making of the present invention has focused the inventors' attention on the fact that CC-RCC may exist as two distinct types: aggressive and non-aggressive, and that this distinction is of prime clinical importance. In the aggressive form, the primary tumor grows more rapidly, tends to metastasize sooner, the metastases grow more rapidly, and patients die sooner. Patients manifesting the aggressive type typically manifest stages III or IV. Non-aggressive RCC, patients typically manifest at stages I or II.

Current diagnosis of CC-RCC is limited to histologic analysis (in addition to corporal imaging, *e.g.*, by ultrasonography, CT scans and X-rays). However, these modalities lack the rigor to distinguish fully between aggressive and non-aggressive tumor phenotype as conceived by the present inventors. Moreover, delays in staging and diagnosis of primary tumors in pre-symptomatic patients narrows the window for successful treatment, particularly of aggressive tumors which may have progressed to metastatic tumor before initial diagnosis.

The marked heterogeneity of CC-RCC provides one of the greatest challenges in diagnosis and treatment. This complicates prognosis and hinders selection of the most appropriate therapy. With the publication of the sequence of the human genome and the advent of high-throughput genomic and proteomic screening technologies, the molecular classification of human cancers are beginning to improve and will surely lead to better diagnosis and more specifically tailored and effective treatment strategies.

Because approximately 30% of CC-RCC patients present with metastatic disease and a short life expectancy (see above) and, of those with initially non-metastatic disease, approximately 30% relapse after surgery, there is an urgent need in the art to identify this latter group of patients before relapse so that appropriate therapies can be offered. To date, no such prognostic tool exists. The present invention provides such a tool for the first time, supplementing the available diagnostic approaches with a genetic screening approach that distinguishes between aggressive and non-aggressive tumor types by the differential expression of certain selected genes, expressed sequence tags (ESTs), gene fragments, mRNAs, and other polynucleotides as described herein.

The present inventors and others (Golub, TR *et al.*, (1999) *Science 286*:531-537; Alizadeh, AA *et al.* (2000) *Nature 403*:503-511; Perou, CM *et al.*, (2000) *Nature 406*:747-752; Bittner, M *et al.*, (2000) *Nature 406*:536-540) have proposed that gene expression profiling using microarray technology can uncover the underlying molecular heterogeneity of cancers, thus identifying new classification schemes and means for more accurate diagnosis and prognosis. Lander's group successfully distinguished between acute myeloid leukemia and acute lymphoblastic leukemia by gene expression profiles (Golub *et al.*, *supra*). Alizadeh *et al.* (*supra*) identified two distinct forms of diffuse large B-cell lymphoma with significantly different prognoses. In these studies, the ability to arrive at a clinically relevant molecular distinction was dependent on known cellular or molecular differences which correlated with gene expression profiles. However, one cannot create a meaningful molecular classification of diseases for which such cellular/molecular information is unavailable. Moreover, many microarray-based gene expression studies have been limited to comparisons of malignant tissue with normal tissue (or related cell lines). Without follow-up clinical data, the most important molecular profiles and relationships may remain obscure. Genetic aberrations common to all CC-RCC (discovered by the present inventors and described herein) may be initial contributing factors to disease. However, the presently disclosed set of differentially expressed DNAs may be responsible for the ultimate course of the disease.

Relevant Genetic Markers

This section provides general information about a number of genes that the present inventors have found to be differentially expressed in CC-RCC of different clinical severity.

The gene for transforming growth factor βII (TGFβII) receptor (TGFβIIR) is of particular interest to this invention as the present inventors have discovered its down-regulation to be

associated with aggressive CC-RCC. The activated TGFβIIR is a heteromeric complex transmembrane protein with intrinsic cytoplasmic serine–threonine kinase domains through which the receptor complex suppresses cellular proliferation via initiation of a tumor suppression pathway. The ligand for this receptor, TGFβ, has three known isoforms in mammals: TGFβ1, TGFβ2, and TGFβ3. These proteins are members of a ligand family for TGFβIIR (which includes activin and bone morphogenic protein).

TGFβs interact with the TGFβIIR which, in turn, recruits the complex formed between TGFβIR and ALK5 to form a heterotetrameric complex. This constitutively activates the TGFβIIR kinase (Markowitz *et al.*, *Cytokine Growth Factor Rev.* (1996) 7:93-102). Other members of the TGFβ superfamily interact with different combinations of homologous type I and type II receptor serine-threonine kinases. The activated kinase phosphorylates TGFβIR at the GS box, a conserved sequence of Gly and Ser residues N-terminal to the kinase domain. A strong correlation exists between malignant progression and loss of sensitivity to the anti-proliferative effects of TGFβ, which is frequently associated with reduced expression or inactivation of TGFβ receptors (Kim *et al.*, *Cytokine Growth Factor Rev* (2000) 11:159–168).

A number of mutations inactivate TGFβIIR. They include truncation at amino acid 97, BAT-RII mutations (big polyadenine tract mutation in exon 3 of TGFβIIR gene), Glu^{142} to stop, and single amino acid substitutions at various positions. BAT-RII is associated with frameshift mutation in a 10-bp polyadenine tract resulting in a truncated receptor that lacks the serine-threonine kinase domain (Markowitz S *et al.*, Science (1995) 268:1336-1338). Receptor mutations, like Thr^{315} to Met, do not interfere with the kinase activity but nevertheless enhance metastatic potential by specifically impeding TGFβ-mediated growth arrest without affecting the induction of extracellular matrix formation (Grady WM *et al.*, (1999) *Cancer Res 59*:320-346).

Whereas TGFβR mutations (other than the BAT-RII frameshift) are rare events in tumorigenesis, repression of TGF-βR expression appears to be a common mechanism enabling tumor cells to escape from negative growth regulation by TGFβ. Mutations inactivating TGFβIIR kinase prevent phosphorylation of Smad family proteins which participate in the tumor suppression pathway. However, a reduction in TGFβIIR signaling in tumor cells is often accompanied by increased expression and secretion of TGFβ which functions independently through its effects on

tumor cells and promotes tumorigenesis and metastasis (Abou-Shady *et al.*, (1999) *Am. J. Surg.* *177*:209-215).

Captopril an inhibitor of angiotensin converting enzyme (ACE) was shown to attenuate growth human CC-RCC xenografts in immunosuppressed mice (Hii, SI *et al.*, (1998) *Br J Cancer* 77:880-883). Though captopril's action and role in tumor suppression is not understood, this molecule is known to up-regulate TGFβIIR expression indirectly (Miyakima A. *et al.*, (2001) *J Urol* **165**:616-620) and to be anti-angiogenic (Volpert OV *et al.*, (1996) *J Clin Invest* 98: 671-679).

Tissue inhibitor of metalloproteinase 3 (TIMP3) is also of interest to the present invention as disclosed below and has been implicated in RCC in previous studies (Kugler, A. *Anticancer Res.* (1999) **19**:1589-1592, Kugler, A., *et al.*, (1996) *J. Urol.* **160**:1914-1918; Lien, M., *et al.*, (2000) *Int. J. Cancer* **85**:801-804). Matrix metalloproteinases (MMPs) are a group of zinc dependant enzymes responsible for extracellular matrix (ECM) degradation. They include type IV collagenases and 92kDa gelatinase (MMP-9). The balance between MMP and available free TIMP (TIMP3 is of interest to the present invention) determines the net MMP activity. The ECM serves as a barrier between endothelial cells and the underlying stroma. Metastatic cancer cells repeatedly cross this barrier in a process requiring proteolysis. Metastasis occurs when the MMP:TIMP ratio exceeds 1 (Kugler, A. *supra*). Conversely, down-regulation or an inactivating mutation in TIMP can also give rise to tumor progression and metastasis.

Kininogens and their cleavage products are conserved multifunctional proteins (Cottrell GA, *et al.*, (1966) *Nature*, **212**: 838-839, Rawlings ND *et al.*, (1990) *J Mol. Evol.* **30**: 60-71). In humans, low molecular weight kininogen (LK, ~65 kDa) and high molecular weight kininogen (HK, ~120 kDa) are single chain glycoproteins made up of kinin domains. Specific hydrolysis by tissue and plasma kallikreins releases Lys-bradykinin (Lys-BK) and bradykinin (BK), respectively, and cleaves each HK into two disulfide-linked fragments (heavy and light chains). Both LK and HK result from alternative splicing of mRNA transcribed from a single 11 exon gene that maps to chromosome 3q26-qter in humans (Fong D *et al.*, (1991) *Human Genetics* **87**:189-192, Takagaki Y *et al.*, (1985) *J Biol. Chem.* **260**:8601-8609). The importance of HK/LK and kinins to normal biologic function is supported by the fact that kininogens and kinins have been conserved through evolution, participate in multiple biologic processes including inflammation, regulation of blood

pressure and vascular permeability, cardioprotection and pain modulation (Rocha *et al.*, (1949) *Amer J Physiol* **156**: 261-273), and by the ubiquity of kinin receptors in mammalian tissues.

SUMMARY OF THE INVENTION

The present inventors set out to characterize CC-RCC at the molecular level by identifying genes whose expression was altered (up or down) in a large percentage of CC-RCC cases. Furthermore, using a clinically well-characterized patient population, they sought to correlate the global gene expression profiling of CC-RCC with tumor progression and clinical outcome, even in the absence of known cellular or molecular characteristics of these tumors.

They hypothesized that by correlating gene expression with clinical parameters, they would uncover a molecular classification scheme for CC-RCC and thus enhance the understanding of progression of this disease. In summary, the objectives were (1) to identify common features of renal cell tumorigenesis, specifically, genes that were regularly up- or down regulated; (2) to generate a molecular portrait of clinically heterogeneous CC-RCC; (3) to identify specific molecular signatures of CC-RCC associated with a particular clinical subset of tumors; and finally, (4) to assess the clinical utility of a particular set of genes as a prognostic tool.

Beyond prognosis and defining new sub-types of disease, the discovery of a set of differentially expressed genes provides a basis for explaining the differences in aggressiveness and clinical outcome. Because genes that best discriminate two phenotypes are expected to be factors in that difference, the clinical follow-up data described herein allows investigation of genes with expression profiles unique to a particular clinical subtype.

Finally, use of the methods and compositions described herein permit identification of (A) proteins whose detection provide an early diagnostic approach to CC-RCC proteins as well (B) drug targets as the products of genes (i) whose expression is commonly altered in CC-RCC or (ii) whose activity is altered in a disease phenotype-selective manner. Thus, by discovering that a particular gene is differentially regulated in aggressive CC-RCC, one can focus on developing drugs that (1) correct down regulation or suppress up-regulation, for example by acting on cellular pathways that stimulate expression of this gene, (2) act directly on the protein product, or (c) bypass the step in a cellular pathway mediated by the product of this gene.

The present inventors have discovered a set of expressed nucleic acid markers through statistical clustering analysis, whose differential expression is indicative of heterogeneous CC-RCC disease manifestation.

The present invention provides a nucleic acid probe or a set of probes (preferably between 2 and 217 in number) and a microarray comprising these DNA markers as probes for the gene expression levels that are characteristic of CC-RCC tumor tissue compared to normal tissue from the same kidney. In one embodiment, the presence and levels of mRNA in a tissue being analyzed are screened using methods known in the art (*i.e.*, Southern/Northern/Western blotting, gel electrophoresis, RFLP, SSCP). The invention is further directed to a method of implementing the microarray technology for disease prognosis (aggressive vs. non-aggressive CC-RCC) thereby supplementing currently available prognostic techniques (radiologic imaging) and pathological classification.

Use of the accurate, objective molecular methods described herein will inform physicians about which patients require heightened observation and additional, *e.g.*, adjuvant, therapies -- for example patients presenting with low stage CC-RCCs that appear on their face to be non-aggressive by conventional criteria, but that have the aggressive type molecular signatures as described herein. Moreover, in the case of patients presenting with higher stage CC-RCCs that might mistakenly be diagnosed as aggressive, but which have the non-aggressive molecular signature, this invention facilitates withholding of unnecessarily aggressive treatment while maintaining appropriate vigilance.

Thus, the present invention is directed to a prognostic microarray composition of at least one oligonucleotide or polynucleotide probe from a set of probes immobilized to a solid surface in a predetermined order such that a row of pixels corresponds to replicates of one distinct probe from the set. The probes are complementary to nucleic acid sequences expressed differentially in aggressive as compared to non-aggressive types of CC-RCC. The probes are preferably any of SEQ ID NO:1 — SEQ ID NO:39 inclusive, SEQ ID NO:139 or SEQ ID NO:332 — SEQ ID NO:497, inclusive. The nucleic acid sequences hybridize to the probes under high stringency conditions.

The microarray may comprise at least about 10 probes, or in another embodiment, at least about 39 or even at least about 206 probes, which probes are complementary to nucleic acid

sequences expressed differentially in aggressive as compared to non-aggressive types of CC-RCC. These probes are preferably at least about 15 nucleotides in length.

The microarray of the present invention can be used to assay expressed nucleic acid samples (representing genes differentially expressed in normal kidney versus CC-RCC tumor tissue) for one or more individual subject's tumor or normal tissue, wherein each sample from an individual subject's tumor or normal tissue is spotted column-wise on the pixels of the microarray probes. The microarray can comprise at least 10, or, in another embodiment, at least about 99, or at least about 291 probes.

In one embodiment, the composition comprises the microarray to which are hybridized and thus immobilized, expressed nucleic acids from the subject. Preferably, hybridization is performed under stringent conditions.

The above microarray probes can comprise nucleotides having at least one modified phosphate backbone, *e.g.*, phosphorothioate, a phosphoridothioate, a phosphoramidothioate, a phosphoramidate, a phosphordiimidate, a methylsphosphonate, an alkyl phosphotriester, 3'-aminopropyl, a formacetal, or analogues thereof.

Also provided is a composition comprising a set of two or more oligonucleotide or polynucleotide probes, each of which hybridizes with part or all of a coding sequence that is differentially expressed in aggressive type CC-RCC compared to non-aggressive type CC-RCC. The above set of probes can comprise at least about 10 probes, or, in another embodiment, at least about 39 probes, or even at least about 206 probes.

The differentially expressed nucleic acid sequences detected by the probes may be ones that are up-regulated or down- regulation in one form of CC-RCC compared to normal tissue or compared to the other form of CC-RCC (aggressive vs. non-aggressive).

The above probes are typically of mammalian, preferably human, origin.

Also provided is a method of predicting whether a subject with a CC-RCC has non-aggressive or aggressive-type CC-RCC. In this method, the expression of nucleic acids from the subject's normal kidney tissue versus kidney tumor tissue is compared in its hybridization, preferably at high stringency conditions, with one or more oligonucleotide or polynucleotide probes as above, preferably probes selected from those having the sequence SEQ ID NO:1 — SEQ ID NO:21 or SEQ ID NO:22 — SEQ ID NO:39.

In one embodiment using probes of the sequence SEQ ID NO:1 — SEQ ID NO:21, up-regulation of at least 2-fold, preferably 3-fold, more preferably 4-fold, in tumor tissue is indicative of non-aggressive CC-RCC.

In another embodiment using probes of the sequence SEQ ID NO:22 — SEQ ID NO:39, down-regulation of at least 2-fold, preferably 3-fold, more preferably 4-fold, in tumor tissue is indicative of aggressive CC-RCC.

In the above methods, the nucleic acids from the tumor and the tissue are detectably labeled, preferably with a fluorescent label prior to the hybridization. With fluorescent labels, hybridization is detected as a fluorescent signal bound to the probe.

In one embodiment of the above method, the probes are immobilized to a solid surface of a microarray as pixels arranged in rows, and the expressed nucleic acids from the tumor tissue or normal tissue samples are spotted column-wise onto the probe pixels.

Also provided is a method for the early diagnosis of a CC-RCC tumor in a subject prior to physical or radiological evidence of the tumor. In this method a protein product of at least one gene is selected based on its expression being up-regulated in a majority of CC-RCC patients. This protein product is preferably a secreted protein or a cell surface protein expressed in tissue readily accessible for assay. The presence or quantity of the protein product in a body fluid or a tissue or cell sample from the subject is determined. An increased level of the protein product compared to the level in a normal subject's fluid, tissue or cells (or another reference normal value) is indicative of the presence of a CC-RCC tumor in the subject.

This invention also provides is a method for diagnosing the recurrence of a CC-RCC tumor in a subject in whom a CC-RCC primary tumor has been excised or otherwise treated. In this method a protein product of at least one gene is selected based on its expression being up-regulated in a majority of CC-RCC patients. This protein product is preferably a secreted protein or a cell surface protein expressed in tissue readily accessible for assay. The presence or quantity of the protein product protein product in a body fluid or a tissue or cell sample from the subject is determined. An increase in the level of the protein product compared to the level in a normal subject's fluid, tissue or cells (or another reference normal value) is indicative of the presence of a recurrent CC-RCC tumor in the subject.

In both methods of early diagnosis and diagnosis of recurrence, the gene is preferably one that hybridizes with any one or more of SEQ ID NO:40 — SEQ ID NO:68 or SEQ ID NO:140 — SEQ ID NO:230, more preferably with one or more of SEQ ID NO:40 — SEQ ID NO:68.

The invention also provides a kit comprising a microarray, reagents that facilitate hybridization of differentially expressed nucleic acid to the immobilized probes on the microarray, and a computer readable storage medium comprising logic which enables a processor to read data representing detection of hybridization. These kits are useful for the diagnosis of aggressive or non-aggressive CC-RCC.

In one embodiment of the provided kit, the reagents facilitate detection of fluorescence as the means for determining hybridization.

Also included is a kit comprising (a) the microarray or composition of any of claims 1-22; (b) means for carrying out hybridization of the nucleic acid to the probes; and (c) means for reading hybridization data. The hybridization data is preferably in the form of fluorescence data. The probes are preferably immobilized to the microarray.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an unsupervised two-way clustering matrix for all 3,184 genes tested. Colored bars on the right represent nodes with high predictive scores. Patient groups appear at the top coded in red, blue or black: Red- poor outcomes; Blue - good outcome; Black - short follow-up period.

Figures 2A and 2B show a supervised two-way re-clustering matrix (Fig. 2A) of independent 'predictive' node 1281, and its respective dendrogram (Fig. 2B) displaying the similarity of patient samples based on a specific subsets of genes. Color code for patients as for Fig. 1. The colors appearing in the multicolor bar beneath the dendrogram appearing at the very bottom of Fig. 2B represent the average expression values for the subsets of genes for each patient.

Figures 3A and 3B show a supervised two-way re-clustering matrix (Fig. 3A) of independent 'predictive' node 3014, and its respective dendrogram (Fig. 3B) displaying the similarity of patient samples based on a specific subsets of genes. Color code for patients as for Fig. 1. The colors appearing in the multicolor bar beneath the dendrogram appearing at the very bottom of Fig. 3B represent the average expression values for the subsets of genes for each patient.

Figures 4A and 4B show a supervised two-way re-clustering matrix (Fig. 4A) of independent 'predictive' node 2199, and its respective dendrogram (Fig. 4B) displaying the similarity of patient samples based on a specific subsets of genes. Color code for patients as for Fig. 1. The colors appearing in the multicolor bar beneath the dendrogram appearing at the very bottom of Fig. 4B represent the average expression values for the subsets of genes for each patient.

Figure 5 shows an expression matrix of a prognostic set of 51 genes (node 1281 from Figs. 2A and 2B). Median centering of genes was not performed so that each square corresponds to the actual normalized gene expression level relative to normal tissue. The red bar labeled "A" marks genes mostly up regulated in low-risk, non-aggressive tumors. The green bar "B" marks genes mostly down regulated in high-risk, aggressive tumors.

Figure 6 is shows clustering expression matrices of subsets of genes the expression of which was detected in 29 CC-RCC tumors. Rows represent individual polynucleotide probes (cDNAs or ESTs) immobilized to the slides; columns represent individual patient tumor samples (as fluorescently labeled cDNAs). Each square's color corresponds to the median-polished, normalized DNA expression value for a single gene in a single tumor relative to patient-matched normal renal tissue. Gene expression is either depicted in RED (above median), GREEN (below median), BLACK (equal to median) or GRAY (inadequate or missing data). The color saturation indicates the extent of divergence from the median. Figures 6A and 6B show supervised two-way re-clustering matrices of three independent 'predictive' nodes (reproduced as enlarged views in Figures 2A, 3A, and 4A). Figure 6C shows the respective dendrograms displaying the similarity of patient samples based on specific subsets of genes (reproduced as enlarged views in Figures 2B, 3B, and 4B). Color code for patients: Red- poor outcomes; Blue - good outcome; Black - short follow-up period. The colors appearing in the multicolor bar beneath each dendrogram (Fig. 6C) represent the average expression values for the subsets of genes for each patient.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the present application, the terms "nucleic acid" and "polynucleotide" are used interchangeably and refer to both DNA and RNA (as well as peptide nucleic acids). The term "oligonucleotide" is not intended to be limited to a particular number of nucleotides and therefor overlaps with polynucleotide. Probes for gene expression analysis include

those comprising ribonucleotides, deoxyribonucleotides, both or their analogues as described below. They may be poly- or oligonucleotides, without limitation of length. Preferred lengths are described below.

The present invention uses cDNA microarrays to probe for, and to determine the relative expression of, target genes of interest in a tissue sample of CC-RCC.

Microarrays are orderly arrangements of spatially resolved samples or probes (in the present invention cDNAs of known sequence ranging in size from 200 to 2000 nucleotides), that allow for massively parallel gene expression and gene discovery studies (Lockhart DJ *et al.*, *Nature* (2000) 405(6788):827-836). The probes are immobilized to a solid substrate and made available to hybridize with their complementary strands as is described in the preferred embodiments (Phimister, *Nature Genetics* (1999) 21(supp):1-60).

The underlying concept of the microarray depends on base-pairing (hybridization) between purine and pyrimidine bases following the rules of Watson-Crick base pairing. Microarray technology adds automation to the process of resolving nucleic acids of particular identity and sequence present in an analyte sample by labeling, preferably with fluorescent labels, and subsequent hybridization to their complements immobilized to a solid support in microarray format. Array experiments employ common solid supports such as glass slides, microplates or standard blotting membranes, and can be created by hand or by robotic deposition of samples. Arrays are generally described as macroarrays or microarrays. Macroarrays contain sample spots of about 300 μm diameter or larger and can be easily imaged by existing gel and blot scanners. Sample spot sizes in microarrays are typically <200 μm in diameter, and these arrays usually contains thousands of spots. Microarrays require specialized robotics and imaging equipment that generally are commercially available and well-known in the art. However, the materials for a particular application are not necessarily available in convenient in kit form. The present invention provides microarrays useful for analysis and prognosis of CC-RCC samples.

DNA microarrays (DNA "chips") are fabricated by high-speed robotics, preferably on glass (though nylon and other plastic substrates are used). An experiment with a single DNA chip can provide simultaneous information on thousands of genes - a dramatic increase in throughput (Reichert *et al.* (2000) *Anal. Chem.72*:6025 –6029) when compared to traditional methods.

Two DNA microarray formats are preferred.

Format I: a cDNA probe (500~5,000 bases) is immobilized to a solid surface such as glass using robotic spotting and exposed to a set of targets either separately or in a mixture. This method, traditionally called "DNA microarray," is considered to have been developed at Stanford University (Ekins, R *et al.*, *Trends in Biotech* (1999) 17:217-218).

Format II: an array of probes that are "natural" oligo- or polynucleotides (oligomers of 20~80 bases), oligonucleotide analogues *e.g.*, with phosphorothioate, methylphosphonate, phosphoramidate, or 3'-aminopropyl backbones), or peptide-nucleic acids (PNA)

Probes may be synthesized either *in situ* (on-chip) or by conventional synthesis followed by on-chip immobilization.

The array is (1) exposed to an analyte comprising a detectable labeled, preferably fluorescent, sample nucleic acid (typically DNA), (2) allowed to hybridize, and (3) the identity and/or abundance of complementary sequences is determined.

1. Probe (cDNA or oligonucleotide of known identity)	**2. Chip** fabrication (putting probes on the chip)	**3. Target** (detectably labeled sample)	**4. Assay**	**5. Readout**
Small oligos, cDNA, chromosome	Photolithography, pipette, drop-touch, piezoelectric (ink-jet), electric	PolyA-mRNA extraction, RT-PCR, cDNA isolation, melting	Hybridization, long, short, ligase, base addition, electric, MS, electrophoresis, flow cytometry, PCR-Direct, TaqMan®, *etc.*	Fluorescence, radioactivity, *etc.*

For analysis of the target nucleic acid of primary tumor tissue, the preferred analyte of this invention is isolated from tissue biopsies before they are stored or from fresh-frozen tumor tissue of the primary tumor which may be stored and/or cultured in standard culture media. For expression studies, poly(A)-containing mRNA is isolated using commercially available kits, *e.g.*, from Invitrogen, Oligotex, or Qiagen. The isolated mRNA is reverse transcribed into cDNA in the presence of a labeled nucleotides. Fluorescent cDNA is generally synthesized using reverse transcriptase (*e.g.*, Superscript II reverse-transcription kit from GIBCO-BRL) and nucleotides to which is conjugated a fluorescent label. A preferred fluorescent label is Cy5 conjugated to dUTP and/or dCTP (from Amersham).

The present invention utilizes immobilized cDNA probes of anywhere between about 15 bases up to a full length cDNA, *e.g.*, about 2000 bases. Preferred probes have about 100 bases. Optimal hybridization conditions (*i.e.*, temperature, pH, ion and salt concentrations, and incubation

time) are dependent on the length of the shortest probes as the limiting step and can be adjusted in a continuous fashion by varying the above parameters as is conventional in the art.

Several probe sequences described herein are cDNAs complementary to genes or gene fragments; some are ESTs. Those skilled in the art will appreciate that the probe of choice for a particular gene can be the full length coding sequence or any fragment thereof having at least about 15 nucleotides. Thus, when the full length sequence is known, the practitioner can select any appropriate fragment of that sequence. When the original results are obtained using partial sequence information (*e.g.*, an EST probe), and when the full length sequence of which that EST is a fragment becomes available (*e.g.*, in a genome database), the skilled artisan can select a longer fragment than the initial EST, as long as the length is at least about 15 nucleotides.

The present invention includes microarrays comprising one or more nucleic acid probes having hybridizable fragments of any length (from about 15 bases to full coding sequence) for the genes whose expression is to be analyzed. For purposes of the analysis, the full length sequence must not necessarily be known, as those of skill in the art will know how to obtain the full length sequences using the sequence of a given EST and known data mining, bioinformatic, and DNA sequencing methodologies without undue experimentation.

The polynucleotide or oligonucleotide probes of the present invention may be native DNA or RNA molecules or an analogues of DNA or RNA. The present invention is not limited to the use of any particular DNA or RNA analogue; rather any one is useful provided that it is capable of adequate hybridization to the complementary DNA (or mRNA) in a test sample, has adequate resistance to nucleases and stability in the hybridization protocols employed. DNA or RNA may be made more resistant to nuclease degradation *in vivo* by modifying internucleoside linkages (*e.g.*, methylphosphonates or phosphorothioates) or by incorporating modified nucleosides (*e.g.*, 2'-0-methylribose or 1'-α-anomers) as described below.

A poly- or oligonucleotide may comprise at least one modified base moiety, for example, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl)uracil, 5-carboxymethylaminomethyl-ω-thiouridine, 5-carboxymethyl-aminomethyl uracil, dihydrouracil, β-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 3-methyl-cytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyamino-methyl-2-thiouracil, β-D-mannosylqueosine, 5-

methoxy-carboxymethyluracil, 5-methoxyuracil-2-methylthio-N6-iso-pentenyladenine, uracil-5-oxyacetic acid, butoxosine, pseudouracil, queuosine, 2-thio-cytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-t-oxyacetic acid, 5-methyl-2-thiouracil, 3(3-amino-3-N-2-carboxypropyl) uracil and 2,6-diaminopurine.

The poly- or oligonucleotide may comprise at least one modified sugar moiety including, but not limited, to arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the poly- or oligonucleotide probe comprises a modified phosphate backbone synthesized from a nucleotide having, for example, one of the following structures: a phosphorothioate, a phosphoridothioate, a phosphoramidothioate, a phosphoramidate, a phosphordiimidate, a methylsphosphonate, an alkyl phosphotriester, 3'-aminopropyl and a formacetal or analog thereof.

In yet another embodiment, the poly- or oligonucleotide probe is an α-anomeric oligonucleotide which forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gautier *et al.*, 1987, *Nucl. Acids Res. 15*:6625-6641).

An oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a hybridization-triggered cleavage agent, *etc.*, all of which are well-known in the art.

Oligonucleotides of this invention may be synthesized by standard methods known in the art, *e.g.* by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, *etc.*). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein *et al.*, (*Nucl. Acids Res.* (1998) *16*:3209, methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* (1988) *85*:7448-7451), *etc.*

Detectable Labels for Oligo- or Polynucleotide Probes

Preferred detectable labels include a radionuclides, fluorescers, fluorogens, a chromophore, a chromogen, a phosphorescer, a chemiluminescer or a bioluminescer. Examples of fluorescers or fluorogens are i fluorescein, rhodamine, dansyl, phycoerythrin, phycocyanin, allophycocyanin, *o*-phthaldehyde, fluorescamine, a fluorescein derivative, Oregon Green, Rhodamine Green, Rhodol Green or Texas Red.

Common fluorescent labels include fluorescein, rhodamine, dansyl, phycoerythrin, phycocyanin, allophycocyanin, *o*-phthaldehyde and fluorescamine. Most preferred are the labels described in the Examples, below.

The fluorophore must be excited by light of a particular wavelength to fluoresce. See, for example, Haugland, *Handbook of Fluorescent Probes and Research Chemicals*, Sixth Ed., Molecular Probes, Eugene, OR., 1996).

Fluorescein, fluorescein derivatives and fluorescein-like molecules such as Oregon Green™ and its derivatives, Rhodamine Green™ and Rhodol Green™, are coupled to amine groups using the isothiocyanate, succinimidyl ester or dichlorotriazinyl-reactive groups. Similarly, fluorophores may also be coupled to thiols using maleimide, iodoacetamide, and aziridine-reactive groups. The long wavelength rhodamines, which are basically Rhodamine Green™ derivatives with substituents on the nitrogens, are among the most photostable fluorescent labeling reagents known. Their spectra are not affected by changes in pH between 4 and 10, an important advantage over the fluoresceins for many biological applications. This group includes the tetramethylrhodamines, X-rhodamines and Texas Red™ derivatives. Other preferred fluorophores are those which are excited by ultraviolet light. Examples include cascade blue, coumarin derivatives, naphthalenes (of which dansyl chloride is a member), pyrenes and pyridyloxazole derivatives.

The present invention serves as a basis for even broader implementation of microarrays and gene expression in deducing critical pathways implicated in cancer. In the case of CC-RCC, which is the focus of the present invention, a database of known patient genetic profiles can be used to categorize each new CC-RCC patient. The gene expression profile of the newly diagnosed CC-RCC patient is compared to the known CC-RCC molecular database of patients, such as that described herein based on 29 patients in whom complete clinical follow-up information is available. This database will grow with each patient who is subjected to the present analysis as soon as his clinical outcome information becomes available. If the newly diagnosed patient's gene expression profile most closely resembles the profile of aggressive CC-RCC, as described herein, that patient will be so classified and treated accordingly, *i.e.*, with more aggressive measures. Correspondingly, if a newly diagnosed patient's profile is that of the non-aggressive type, he will be treated accordingly, *e.g.*, with less aggressive measures and careful clinical follow-up.

Considering the low response rates of CC-RCC patients to current therapies such as with interferon-α and interleukin-2 infusion, the report that the apoptosis following induction of TIMP3 (Ahonen, *et al.* (1998) *Cancer Res* 58:2310-2315) coupled with the discovery here that TIMP3 is down-regulated in aggressive CC-RCC points to a new potential therapeutic strategy that may include gene therapy. The present approach permits the identification of one or more appropriate targets for such therapy.

Drug Discovery Based on Gene Expression Profiling

The molecular profiling information described herein is also harnessed for the purpose of discovering drugs that are selected for their ability to correct or bypass the molecular alterations or derangements that are characteristic of CC-RCC, particularly those that are associated with its aggressive form. A number of approaches are available.

In one embodiment, CC-RCC cell lines are prepared from tumors using standard methods and are profiled using the present methods. Preferred cell lines are those that maintain the expression profile of the primary tumor from which they were derived. One or several CC-RCC cells lines may be used as a "general" panel; alternatively or additionally, cell lines from individual patients may be prepared and used. These cell lines are used to screen compounds, preferably by high-throughput screening (HTS) methods, for their ability to alter the expression of selected genes. Typically, small molecule libraries available from various commercial sources are tested by HTS protocols.

The molecular alterations in the cell line cells can be measured at the mRNA level (gene expression) applying the methods disclosed in detail herein. Alternatively, one may assay the protein product(s) of the selected gene(s). Thus, in the case of secreted or cell-surface proteins, expression can be assessed using immunoassay or other immunological methods including enzyme immunoassays (EIA), radioimmunoassay (RIA), immunofluorescence microscopy or flow cytometry. EIAs are described in greater detail in several references (Butler, JE, In: *Structure of Antigens*, Vol. 1 (Van Regenmortel, M., CRC Press, Boca Raton 1992, pp. 209-259; Butler, JE, "ELISA," In: van Oss, C.J. *et al.* (eds), *Immunochemistry*, Marcel Dekker, Inc., New York, 1994, pp. 759-803; Butler, JE (ed.), *Immunochemistry of Solid-Phase Immunoassay*, CRC Press, Boca Raton, 1991). RIAs are discussed in Kirkham and Hunter (eds.), *Radioimmune Assay Methods*, E. & S. Livingstone, Edinburgh, 1970.

In another approach, antisense RNAs or DNAs that specifically inhibit the transcription and/or translation of the targeted genes can be screened for specificity and efficacy using the present methods. Antisense compositions would be particularly useful for treating tumors in which a particular gene is up-regulated (*e.g.*, the genes in Tables 2 and 3).

Diagnostic Methods

The protein products of genes that are upregulated in most cases of CC-RCC (*e.g.*, Tables 2 and 3) are targets for early diagnostic assays of CC-RCC if the proteins can be detected by some assay means, *e.g.*, immunoassay, in some accessible body fluid or tissue. The most useful diagnostic targets are secreted proteins which reach a measurable level in a body fluid before the tumor presents by other criteria discussed in the Background section. Thus, a sample of a body fluid such as such as plasma, serum, urine, saliva, cerebrospinal fluid, etc., is obtained from the subject being screened. The sample is subject to any known assay for the protein analyte. Alternatively, cells expressing the protein on their surface may be obtained, *e.g.*, blood cells, by simple, conventional means. If the protein is a receptor or other cell surface structure, it can be detected and quantified by well-known methods such as flow cytometry, immunofluorescence, immunocytochemistry or immunohistochemistry, and the like.

Preferably, an antibody or other protein or peptide ligand for the target protein to be detected is used. In another embodiment where the gene product is a receptor, a peptidic or small molecule ligand for the receptor may be used in known assays as the basis for detection and quantitation.

In vivo methods with appropriately labeled binding partners for the protein targets, preferably antibodies, may also be used for diagnosis and prognosis, for example to image occult metastatic foci or for other types of *in situ* evaluations. These methods utilize include various radiographic, scintigraphic and other imaging methods well-known in the art (MRI, PET, *etc.*).

Suitable detectable labels include radioactive, fluorescent, fluorogenic, chromogenic, or other chemical labels. Useful radiolabels, which are detected simply by gamma counter, scintillation counter or autoradiography include ^{3}H, ^{125}I, ^{131}I, ^{35}S and ^{14}C.

Common fluorescent labels include fluorescein, rhodamine, dansyl, phycoerythrin, phycocyanin, allophycocyanin, *o*-phthaldehyde and fluorescamine. The fluorophore, such as the dansyl group, must be excited by light of a particular wavelength to fluoresce. See, Haugland, *Handbook of Fluorescent Probes and Research Chemicals*, Sixth Ed., Molecular Probes, Eugene,

OR., 1996). Fluorescein, fluorescein derivatives and fluorescein-like molecules such as Oregon Green™ and its derivatives, Rhodamine Green™ and Rhodol Green™, are coupled to amine groups using the isothiocyanate, succinimidyl ester or dichlorotriazinyl-reactive groups. Fluorophores may also be coupled to thiols using maleimide, iodoacetamide, and aziridine-reactive groups. The long wavelength rhodamines include the tetramethylrhodamines, X-rhodamines and Texas Red™ derivatives. Other preferred fluorophores for derivatizing the protein binding partner are those which are excited by ultraviolet light. Examples include cascade blue, coumarin derivatives, naphthalenes (of which dansyl chloride is a member), pyrenes and pyridyloxazole derivatives.

The protein (antibody or other ligand) can also be labeled for detection using fluorescence-emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the protein using metal chelating groups such as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

For *in vivo* diagnosis, radionuclides may be bound to protein either directly or indirectly using a chelating agent such as DTPA and EDTA which is chemically conjugated, coupled or bound (which terms are used interchangeably) to the protein. The chemistry of chelation is well known in the art. The key limiting factor on the chemistry of coupling is that the antibody or ligand must retain its ability to bind the target protein. A number of references disclose methods and compositions for complexing metals to macromolecules including description of useful chelating agents. The metals are preferably detectable metal atoms, including radionuclides, and are complexed to proteins and other molecules. See, for example, US 5,627,286, US 5,618,513, US 5,567,408, US 5,443,816, US 5,561,220, all of which are incorporated by reference herein.

Any radionuclide having diagnostic (or therapeutic value) can be used. In a preferred embodiment, the radionuclide is a γ -emitting or β-emitting radionuclide, for example, one selected from the lanthanide or actinide series of the elements. Positron-emitting radionuclides, *e.g.* ^{68}Ga or ^{64}Cu, may also be used. Suitable γ-emitting radionuclides include those which are useful in diagnostic imaging applications. The gamma -emitting radionuclides preferably have a half-life of from 1 hour to 40 days, preferably from 12 hours to 3 days. Examples of suitable γ-emitting radionuclides include ^{67}Ga, ^{111}In, ^{99m}Tc, ^{169}Yb and ^{186}Re. Examples of preferred radionuclides (ordered by atomic number) are ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{72}As, ^{89}Zr, ^{90}Y, ^{97}Ru, ^{99}Tc, ^{111}In, ^{123}I, ^{125}I, ^{131}I,

^{169}Yb, ^{186}Re, and ^{201}Tl. Though limited work have been done with positron-emitting radiometals as labels, certain proteins, such as transferrin and human serum albumin, have been labeled with ^{68}Ga,

A number of metals (not radioisotopes) useful for MRI include gadolinium, manganese, copper, iron, gold and europium. Gadolinium is most preferred. Dosage can vary from 0.01 mg/kg to 100 mg/kg.

In situ detection of the labeled protein may be accomplished by removing a histological specimen from a subject and examining it by microscopy under appropriate conditions to detect the label. Those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

The compositions of the present invention may be used in diagnostic, prognostic or research procedures in conjunction with any appropriate cell, tissue, organ or biological sample of the desired animal species. By the term "biological sample" is intended any fluid or other material derived from the body of a normal or diseased subject, such as blood, serum, plasma, lymph, urine, saliva, tears, cerebrospinal fluid, milk, amniotic fluid, bile, ascites fluid, pus and the like. Also included within the meaning of this term is a organ or tissue extract and a culture fluid in which any cells or tissue preparation from the subject has been incubated.

An alternative diagnostic approach utilizes cDNA probes that are complementary to and thereby detect cells in which a gene associated with CC-RCC is upregulated by *in situ* hybridization with mRNA in these cells. The present invention provides methods for localizing target mRNA in cells using fluorescent in situ hybridization (FISH) with labeled cDNA probes having a sequence that hybridizes with the mRNA of an upregulated gene. The basic principle of FISH is that DNA or RNA in the prepared specimens are hybridized with the probe nucleic acid that is labeled non-isotopically with, for example, a fluorescent dye, biotin or digoxigenin. The hybridized signals are then detected by fluorimetric or by enzymatic methods, for example, by using a fluorescence or light microscope. The detected signal and image can be recorded on light sensitive film.

An advantage of using a fluorescent probe is that the hybridized image can be readily analyzed using a powerful confocal microscope or an appropriate image analysis system with a charge-coupled device (CCD) camera. As compared with radioactive methods, FISH offers increased sensitivity. In additional to offering positional information, FISH allows better

observation of cell or tissue morphology. Because of the nonradioactive approach, FISH has become widely used for localization of specific DNA or mRNA in a specific cell or tissue type.

The *in situ* hybridization methods and the preparations useful herein are describe in Wu, W. *et al.*, eds., *Methods in Gene Biotechnology*, CRC Press, 1997, chapter 13, pages 279-289. This book is incorporated by reference in its entirety, as are the references cited therein. A number of patents and papers that describe various *in situ* hybridization techniques and applications, also incorporated by reference, are: 5,912,165; 5,906,919; 5,885,531; 5,880,473; 5,871,932; 5,856,097; 5,837,443 ; 5,817,462; 5,784,162; 5,783,387 ; 5,750,340; 5,759,781; 5,707,797; 5,677,130; 5,665,540; 5,571,673; 5,565,322; 5,545,524 ; 5,538,869; and 5,501,954, 5,225,326, 4,888,278. Other related references include Jowett, T, *Methods Cell Biol*;59:63-85 (1999) Pinkel *et al.*, *Cold Spring Harbor Symp. Quant. Biol. LI*:151-157 (1986); Pinkel, D. *et al.*, *Proc. Natl. Acad. Sci. (USA) 83*:2934-2938 (1986); Gibson *et al.*, *Nucl. Acids Res. 15*:6455-6467 (1987); Urdea *et al.*, *Nucl. Acids Res. 16*:4937-4956 (1988); Cook *et al.*, *Nucl. Acids Res. 16*:4077-4095 (1988); Telser *et al.*, *J. Am. Chem. Soc. 111*:6966-6976 (1989); Allen *et al.*, *Biochemistry 28*:4601-4607 (1989); Nederlof, P.M. *et al.*, *Cytometry 10*:20-27 (1989); Nederlof, P.M. *et al.*, *Cytometry 11*:126-131 (1990); Seibl, R., *et al.*, *Biol. Chem. Hoppe-Seyler 371*:939-951 (Oct. 1990); Wiegant, J. *et al.*, *Nucl. Acids Res. 19*:3237-3241 (1991); McNeil JA *et al.*, *Genet Anal Tech Appl 8*:41-58 (1991); Komminoth *et al.*, *Diagnostic Molecular Biology 1*:85-87 (1992); Dauwerse, JG *et al.*, *Hum. Mol. Genet. 1*:593-598 (1992); Ried, T. *et al.*, Proc. *Natl. Acad. Sci. (USA) 89*:1388-1392 (1992); Wiegant, J. *et al.*, *Cytogenet. Cell Genet. 63*:73-76 (1993); Glaser, V., *Genetic. Eng. News.. 16*:1, 26 (1996); Speicher, MR, *Nature Genet. 12*:368-375 (1996).

Detection of "Unknown" Gene Product

In an extreme case, in which an upregulated DNA "X" is identified but its protein product "Y" is unknown, one would first examine the expressed DNA X sequence. The full length gene sequence may be obtained by accessing a human genomic database such as that of Celera. In either case, examination of the coding sequence for appropriate motifs will indicate whether the encoded protein Y is secreted protein or a transmembrane protein. If no antibodies specific for protein Y are already available, the peptides of protein Y can be designed and synthesized using known principles of protein chemistry and immunology. The object is to create a set of immunogenic peptides that elicit antibodies specific for epitopes of the protein that reside on its surface. Alternatively, the coding DNA or portions thereof can be expression-cloned to produce a polypeptide or peptide

epitope thereof. That protein or peptide can be used as an immunogen to immunize animals for the production of antisera or to prepare monoclonal antibodies (mAbs). These polyclonal sera or mAbs can then be applied in an immunoassay, preferably an EIA, to detect the presence of protein Y or measure its concentration in a body fluid or cell/tissue sample.

Therapeutic Methods

Taking the lead from the drug discovery methods described above, one can exploit the present invention to treat CC-RCC based on the knowledge of the genes that are either up- or down-regulated in a highly predicable manner across CC-RCC cases (see Tables 2 -5 in Examples) . Based on the nature of the deduced protein product, one can devise a means to inhibit the action of, or remove an upregulated protein. In the case of a receptor, one would treat the upregulated receptor with an antagonist, a soluble receptor or a "decoy" ligand binding site of a receptor (Gershoni JM *et al.*, *Proc Natl Acad Sci USA,* 1988 85:4087-9; US Patent 5,770,572).

For an under-expressed receptor, an agonist or mimetic would be administered to maximize binding and activation of those receptor molecules which are expressed.

As for the set of genes that are shown here to be down-regulated in aggressive CC-RCC, one can devise a therapy targeted specifically at this form of the cancer, that would be used alone or in combination with known therapeutic approaches as discussed above. A preferred approach would be to stimulate production of the protein by administering an agent that promoted production, enhanced its stability or inhibited its degradation or metabolism. Alternatively, one could design means to bypass the metabolic step or signal pathway step that was affected by this down-regulation. This could by achieved by stimulating downstream steps in such pathways. If a receptor was involved, then, as above agonists or mimics could be used to heighten responses of cells expressing too little of the receptor.

Antibodies may be administered to a patient to bind and inactivate (or compete with) secreted protein products or expressed cell surface products of upregulated genes.

Moreover, for the down-regulated genes, gene therapy methods could be used to introduce more copies of the affected gene or more actively expressed genes operatively linked to strong promoters, *e.g.*, inducible promoters, such as an estrogen inducible system (Braselmann, S. *et al. Proc Natl Acad Sci USA* (1993) *90*:1657-1661). Also known are repressible systems driven by the

conventional antibiotic, tetracycline (Gossen, M. *et al.*, *Proc. Natl. Acad. Sci. USA 89*:5547-5551 (1992)).

In the case of upregulated genes, this approach would be extended to include antisense oligonucleotide or polynucleotide constructs that would inhibit gene expression in a highly specific manner. Multiple antisense constructs specific for different upregulated genes could be employed together. The sequences of the upregulated genes described herein are used to design the antisense oligonucleotides (Hambor, JE *et al.*, *J. Exp. Med. 168*:1237-1245 (1988); Holt, JT *et al.*, *Proc. Nat'l. Acad. Sci. 83*:4794-4798 (1986); Izant, JG *et al.*, *Cell 36*:1007-1015 (1984); Izant, JG *et al.*, *Science 229*:345-352 (1985) ; De Benedetti, A. *et al.*, *Proc. Natl. Acad. Sci. USA 84*:658-662 (1987)). The antisense oligonucleotides may range from 6 to 50 nucleotides, and may be as large as 100 or 200 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotides can be modified at the base moiety, sugar moiety, or phosphate backbone (as discussed above). The oligonucleotide may include other appending groups such as peptides, or agents facilitating transport across the cell membrane (see, *e.g.* Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. USA 84*:684-652; PCT Publication WO 88/09810, published December 15, 1988) or blood-brain barrier (*e.g.*, PCT Publication No. WO 89/10134, published April 25, 1988), hybridization-triggered cleavage agents (*e.g.* Krol *et al.*, 1988, BioTechniques 6:958-976) or intercalating agents (*e.g.*, Zon, 1988, *Pharm. Res 5*:539-549).

The therapeutic methods that require gene transfer and targeting may include virus-mediated gene transfer, for example, with retroviruses (Nabel, E.G. *et al.*, *Science 244*:1342 (1989), lentiviruses, recombinant adenovirus vectors (Horowitz, M.S., In: *Virology*, Fields, BN *et al.*, eds, Raven Press, New York, 1990, p. 1679, or current edition; Berkner, KL, *Biotechniques 6*:616 919,1988), Strauss, SE, In: *The Adenoviruses*, Ginsberg, HS, ed., Plenum Press, New York, 1984, or current edition), Adeno-associated virus (AAV) is also useful for human gene therapy (Samulski, RJ *et al.*, *EMBO J. 10*:3941 (1991); (Lebkowski, JS, *et al.*, *Mol. Cell. Biol.* (1988) 8:3988-3996; Kotin, RM *et al.*, *Proc. Natl. Acad. Sci. USA* (1990) 87:2211-2215); Hermonat, PL, *et al.*, *J. Virol.* (1984) *51*:329-339). Improved efficiency is attained by the use of promoter enhancer elements in the plasmid DNA constructs (Philip, R. *et al.*, *J. Biol. Chem.* (1993) 268:16087-16090).

In addition to virus-mediated gene transfer *in vivo*, physical means well-known in the art can be used for direct gene transfer, including administration of plasmid DNA (Wolff *et al.*, 1990, *supra*) and particle-bombardment mediated gene transfer, originally described in the transformation of plant tissue (Klein, TM *et al.*, *Nature 327*:70 (1987); Christou, P. *et al.*, *Trends Biotechnol. 6*:145 (1990)) but also applicable to mammalian tissues *in vivo*, *ex vivo* or *in vitro* (Yang, N.-S., *et al.*, *Proc. Natl. Acad. Sci. USA 87*:9568 (1990); Williams, RS *et al.*, *Proc. Natl. Acad. Sci. USA 88*:2726 (1991); Zelenin, AV *et al.*, *FEBS Lett. 280*:94 (1991); Zelenin, AV *et al.*, *FEBS Lett. 244*:65 (1989); Johnston, S.A. *et al.*, *In Vitro Cell. Dev. Biol. 27*:11 (1991)). Furthermore, electroporation, a well-known means to transfer genes into cell *in vitro*, can be used to transfer DNA molecules according to the present invention to tissues *in vivo* (Titomirov, AV *et al.*, *Biochim. Biophys. Acta 1088*:131 ((1991)).

Gene transfer can also be achieved using "carrier mediated gene transfer" (Wu, CH *et al.*, *J. Biol. Chem. 264*:16985 (1989); Wu, GY *et al.*, *J. Biol. Chem. 263*:14621 (1988); Soriano, P *et al.*, *Proc. Natl. Acad. Sci. USA 80*:7128 (1983); Wang, C-Y. *et al.*, *Proc. Natl. Acad. Sci. USA 84*:7851 (1982); Wilson, J.M. *et al.*, *J. Biol. Chem. 267*:963 (1992)). Preferred carriers are targeted liposomes (Nicolau, C. *et al.*, *Proc. Natl. Acad. Sci. USA 80*:1068 (1983); Soriano *et al.*, *supra*) such as immunoliposomes, which can incorporate acylated monoclonal antibodies into the lipid bilayer (Wang *et al.*, *supra*), or polycations such as asialoglycoprotein/polylysine (Wu *et al.*, 1989, *supra*). Liposomes have been used to encapsulate and deliver a variety of materials to cells, including nucleic acids and viral particles (Faller, DV *et al.*, J. Virol. (1984) 49:269-272).

Preformed liposomes that contain synthetic cationic lipids form stable complexes with polyanionic DNA (Felgner, PL, et al., *Proc. Natl. Acad. Sci. USA* (1987) 84:7413-7417). Cationic liposomes, liposomes comprising some cationic lipid, that contained a membrane fusion-promoting lipid dioctadecyldimethyl-ammonium-bromide (DDAB) have efficiently transferred heterologous genes into eukaryotic cells (Rose, JK et al., *Biotechniques* (1991) 10:520-525). Cationic liposomes can mediate high level cellular expression of transgenes, or mRNA, by delivering them into a variety of cultured cell lines (Malone, R., et al., *Proc. Natl. Acad. Sci. USA* (1989) 86:6077-6081).

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLE I

Patients and Tumor Samples

Tissue samples were from 29 CC-RCC patients at the University Hospital, School of Medicine, Tokushima University (Japan) who underwent radical nephrectomy. Informed consent was obtained for study of surgical specimens and clinico-pathological data. Samples were anonymized prior to the study. A part of each tumor sample was frozen in liquid nitrogen immediately following surgery and stored at -80°C.

Conventional methods were used for nucleic acid isolation and preparation. Total RNA was isolated using ISOGEN solution (Nippon Gene), and poly(A)+ RNA was isolated from total RNA using the Oligotex mRNA Mini Kit (Qiagen). Remaining tumor tissue was fixed in 10% buffered formalin, sectioned and stained with hematoxylin and eosin. The WHO International Histological Classification of Tumors was used for histological evaluation of the specimens (Sobin, L.H. *et al.*, *supra*)(TNM classification described above) with standard follow up for 3.2 to 137.2 months (median 83.7 months). Clinico-pathological data are summarized in Table 1.

EXAMPLE II

Materials and Methods

Microarray Design

Microarrays were produced using conventional methods and materials well known in the art (Eisen *et al.*, *Methods Enzymol* (1999) **303**:179-205) with slight modifications. Bacterial libraries purchased from Research Genetics, Inc. were the source of 21,632 cDNAs which were PCR amplified 21,632 directly. cDNA clones were ethanol-precipitated and transferred to 384-well plates from which they were printed onto poly-l-lysine coated glass slides using a home-built robotic microarrayer (www.microarrays.org/pdfs/PrintingArrays). The boundaries of the array where then marked with a diamond scriber to discriminate the edges (diamond scriber available by catalogue, VWR #52865-005) since the array is mostly invisible after post-processing. The printed array was immersed into a humid chamber prepared with 100 ml 1X SSC and allowed to rehydrate on an inverted heat block of preferably, 70-80°C, block for about 3 seconds. The cDNA was UV crosslink to glass with Stratalinker set for about 65 mJ. (Preferably, set display to "650", which is 650 x 100 μJ).

Table1. Patient clinical data and corresponding prognosis classifications

					Prognosis Group		
patient	Grade	Stage	Outcome	Duration	Outcome Group	Pathology/ Staging	Gene Expression
46	G1	S1	NED	62.6	L	L	L
42	G1	S1	NED	77.3	L	L	L
41	G1	S1	NED	80.3	L	L	L
30	G2	S3	NED	87.1	L	H*	H*
7	G1	S1	NED	92.1	L	L	L
26	G1	S1	NED	96	L	L	L
24	G1	S1	NED	97.3	L	L	L
15	G1	S1	OCD	100.4	L	L	L
32	G1	S2	OCD	110.4	L	L	L
1	G1	S1	NED	111.6	L	L	L
21	G1	S1	NED	114.6	L	L	L
20	G1	S1	NED	115.8	L	L	L
35	G1	S3	NED	120.5	L	H*	L
9	G1	S3	NED	120.9	L	H*	L
3	G1	S1	NED	137.2	L	L	L
29	G3	S3	AWC	89.4	L	H*	L
54	G1	S4	AWC	105.6	L	H*	L
13	G3	S4	Death	3.2	H	H	H
48	G2	S4	Death	4.9	H	H	H
11	G3	S3	Death	18.8	H	H	H
60	G3	S4	Death	20.8	H	H	H
31	G3	S3	Death	22.6	H	H	H
53	G3	S4	Death	26.2	H	H	H
5	G2	S4	Death	31.7	H	H	H
12	G2	S4	Death	33.8	H	H	H
55	G2	S2	Death	55.8	H	L*	H
56	G3	S4	AWC	14.8	U	H	L
58	G3	S4	AWC	16.6	U	H	H
59	G2	S3	NED	41.1	U	H	H

Stage and grade information (columns 2, 3) is for primary tumor upon resection.
Outcomes (column 4) are: "no evidence of disease at last visit" (NED), "alive with cancer" (AWC), "other cause of death" (OCD) and "death" (due to cancer).
Duration (column 5) is months between nephrectomy and latest outcome assessment.
Outcome group (column 6) is the risk group based on actual patient outcome;
Pathology prognosis group (column 7) is based on staging of primary tumor;
Gene expression prognosis group (column 8) is based on molecular prognosis test based on genes in NODE 1281. Risk groups include high-risk (H), low-risk (L) and unknown (U). * indicates deviation from actual risk group.

for 20 minutes with the lid down. The array was then snap centrifugation dried (cDNA side up)

Prior to applying hybridization solution containing labeled probes (*below*), slides were blocked before target hybridization, using bovine serum albumin (BSA) solution (1% BSA, 5X SSC, 0.1% SDS) as described by Volpert *et al., J Clin Invest* (1999) **98**(3): 671-679). , Blocking is preferably done within 1 hour of hybridization, most preferably immediately before.

Tissue cDNA Preparation

Samples (2μg of poly(A)-RNA from each kidney tumor and from normal kidney tissue from the same patient were reverse transcribed with oligo (dT) primers and Superscript II (Life Technologies, Inc) in the presence of Cy5-dCTP and Cy3-dCTP (Amersham Pharmacia Biotech), respectively (*Methods Enzymol* (1999) **303**:179-205).

The poly(A)-mRNA isolation procedure used by the inventors is detailed below, however, the skilled artisan will appreciate that any method of isolation and fluoro-labeling can be used. The inventors mixed 2 μg of mRNA with 2 μg of a regular or anchored oligo-dT primer in a total volume of 15μl:

	Cy3	Cy5	
mRNA	2 μg	2 μg	
Oligo-dT	2 μg	2 μg	(Anchored: 5'-TTT TTT TTT TTT TTT TTT TTV N-3')* SEQ ID NO: 498
Total volume:	15 μL	μL	

* ("V" refers to A, G or C; "N" refers to A, G, C or T)

Next, the reaction mixture was heated to 70°C for about 10 min and cooled on ice to which was added 15 μL of the following reaction mixture with denatured mRNA (for a total of 30 μl):

Reaction mixture*:	Vol μl
5X first-strand buffer**	6.0
0.1M DTT	3.0
Unlabeled dNTPs	0.6
Cy3 or Cy5-dCTP (1 mM, Amersham)	3.0
Superscript II (200 U/μL, Gibco BRL)	2.0
H_2O	0.4
Total volume:	15

Unlabeled dNTPs 100 mM	Vol μl	Final conc.
dATP	25	25 mM
dCTP	15	15 mM
dGTP	25	25 mM
dTTP	25	25 mM
H_2O	10	

Total volume:	100	

*Reaction mixture (Master Mix) available that contains buffer, DTT, dNTPs, and H_2O (combine 10μl Master Mix with 3μl Cy3 or Cy5 dye and 2μl Superscript)

** 5X first-strand buffer: 250 mM Tris-HCl (pH 8.3), 375mM KCl, 15mM MgCl2)

The combined reaction mix was incubated at 42°C for 1.5-2hrs. RNA degradation was facilitated by the addition of 15μl of 0.1 M NaOH, and incubation at 70°C for 10 min. The degradation

reaction was neutralized by addition of 15µl of 0.1 M HCl, and the total volume was brought to 500µl with TE (10mM Tris, 1mM EDTA).

Next, 20 µg of Cot-1 human DNA (GIBCO-BRL) was added to each sample. The target cDNAs (post RT-PCR replicons) were purified by centrifuging in a Microcon-30 micro-concentrator (Amicon, 10,000 x g (rcf)for 10 min until ~10 µl remained). Purification can be monitored by observing the concentration of the "colored probe."

TE, 450 µl was added to each Microcon-30 unit and the retentate collected in a fresh microtube. The collected retentate from the previous step was added into the Microcon-30 unit containing the other sample in order to combine the separate probes (Cy3 and Cy5). The final volumes should be about 500µl (if less than 500µl, adjust with TE).

The 500µl mix was spun with a microcon-30 (10,000 x g (rcf) x 12 min) containing labeled samples in order to concentrate again to a volume of less than 11µl. Add 1 µL of 10 µg/µl polyA RNA (Sigma, #P9403) and 1 µl of 10 µg/µl tRNA (GIBCO-BRL, #15401-011) and adjust volume to 15-17 µl with distilled water.

The mixture was heated at 95°C for 3 min. and briefly centrifuged to collect condensation. Then the denatured target was combined with equal volume of 2X hybridization solution preheated to 42°C. The mixture was heated at 95°C for 3 min. and briefly centrifuged to collect condensation. Then the denatured target was combined with equal volume of 2X hybridization solution preheated to 42°C.

Hybridizing Microarrays Blocked with BSA

Immediately or shortly before hybridization the prepared microarray slides containing single stranded cDNA probes were BSA blocked (*supra*).

The 2X hybridization solution contains: 50% formamide; 10X SSC; 0.2% SDS. Final volume was 30-35 µl. The hybridization solution was incubated at 42°C for 20-30 min. The labeled target + hybridization solution was then applied to a prepared microarray slide at 42°C (using a hot block to preheat the slide and coverslip).

20µl H_2O was placed in the wells of the hybridization chamber. The slide was sealed in a hybridization chamber and placed in a 42°C water bath. Microarrays were hybridized for ~16-20 hours.

Slides were removed from hybridization chamber and immediately placed in a first rinse station with wafer holders/forceps (5 total wash/rinse stations): Exposure of labeled probe to light is to be minimized. The rinsing protocol is detailed below:

A. 1X SSC, 0.1% SDS (376 mls dH_2O, 20 mls 20X SSC, 4 mls 10% SDS)
This first rinse is carried out at 42°C until the cover slip is washed off, keep the slide in this solution for 5 minutes. Place slide in new metal tray in the next station.

B. 0.2X SSC, 0.1% SDS (392 mls dH_2O, 4 mls 20X SSC, 4 mls 10% SDS)
Gently shake station with slides and holder on rotator for 5 minutes. Take individual slides out of the tray and place in next clean station.

C. 0.2X SSC (396 mls dH_2O, 4 mls 20X SSC)
Shake gently for 5 minutes. Transfer entire slide holder into next station. Carry out this step three times, using fresh solution each time. Preferably, three stations are used where repeated washing steps are carried out with fresh solution.

Slides were dried by snap centrifugation (5 min. at 550 rpm) and scanned immediately using a commercially available confocal fluorescent scanner equipped with lasers operating at 532 nm and 635 nm wavelengths. (Scan Array Lite, GSI Lumonics).

Data analysis

Images were analyzed using the software Genepix Pro 3.0 (Axon). Spots showing no signal or obvious defects were excluded from the analysis. Hybridization signal intensities from the remaining spots had the background subtracted and were tabulated in a red-to-green ratio, representing tumor mRNA expression relative to the mRNA expression of the corresponding normal kidney tissue. Ratios were log transformed and normalized so that the average ratio equaled zero. cDNAs with non-flagged spots in 75% of the experiments and with expression ratios that varied at least 2-fold in at least 2 experiments were selected for further analysis. The ratios were median-polished as described to provide values relative to the other samples. The software programs CLUSTER and TREEVIEW were used for hierarchical clustering and visualization (http://rana.standford.edu/software).

CLUSTERFINDER

The present inventors developed the program "CLUSTERFINDER" to identify sub-clusters of polynucleotides that best distinguish between two defined sample groups. This clustering methodology entails, averaging the polynucleotides within a subcluster so that each patient has one expression value per subcluster. These expression value averages are separated into two groups based on the user-defined criteria. Here, staging criteria and patient fatality were employed. For each group of expression value averages, means (μ) and standard deviations (σ) were calculated. The discrimination score (**ds**) is calculated as follows:

$$\mathbf{ds} = |\mu_1 - \mu_2| / (\sigma_1 + \sigma_2)$$

This metric maximizes difference between the means of the two groups and minimizes the variation within groups (Golub *et al., supra*). The method begins with the smallest clusters (2 cDNAs) and moves through a dendrogram identifying nodes in the tree that maximize both discrimination score and cluster size.

A permuted t-test was used to assess each cDNAs individual ability to distinguish between the two groups of patients (Hedenfalk, I., *et al.* (2001) *N Engl J Med* 344:539-48). Patients were randomly assigned into two groups 10,000 times. For each random permutation, a t-statistic was generated to test expression significance for each cDNA. The distribution of t-statistics was used to define a 99.9% significance threshold (α= 0.001). If the t-statistic for the real distinction exceeded the 99.9% significance threshold, the cDNA was considered predictive.

This design permitted two valuable approaches to analyze the data. First, the use of the patient-matched normal tissue as a reference, against which mRNA expression in the tumors is measured, allows identification of aberrant polynucleotide expression (up or down) in each tumor. Second, since Cy3-labeled normal tissue was a common reference in all the experiments, values obtained from different experiments could be compared directly to identify gene expression patterns that would account for clinical differences such as grade, stage or aggressiveness of the tumor.

EXAMPLE III

Identification of Useful Probes for Up- and Down-Regulated Genes

The inventors first sought to identify genes that were up- or down-regulated regularly in tumor tissue relative to matched normal kidney tissue. The criterion for a useful probe was one that detected a gene that is up-regulated or down-regulated at least 2-fold in at least 75 % of the CC-RCC samples. The inventors identified 129 clones (up) and 168 clones (down) respectively. See Tables 2-5. Up-regulated genes included many notable coding sequences:

(1) ceruloplasmin, (2) an EST highly similar to growth factor responsive protein, (3) nicotinamide N-methyltransferase, (4) lysyl oxidase, (5) an EST highly similar to angiopoietin-related protein,	(6) tumor necrosis factor α-induced protein 6, (7) insulin-like growth factor binding protein-3 (8) enolase-2, (9) fibronectin-1 and (10) vascular endothelial growth factor (VEGF).
Down-regulated cDNAs included:	
(1) kininogen, (2) fatty acid binding protein 1, (3) phenylalanine hydroxylase,	(4) epidermal growth factor, and (5) plasminogen.

In addition, six members of the metallothionein family were down regulated and coordinately expressed across all patients.

Table 2. First Set of Commonly Up-Regulated Genes in CC-RCC

	GenBank Accession #	Name (from Research Genetics database)	SEQ ID NO:	Average Fold Up	Incidence % of RCC*		Celera NO#	E value
					2-fold *	3-fold**		
1	H86554	Ceruloplasmin (ferroxidase)	40	16.9	96.2	96.2	hCG21213	6×10^{-85}
2	R00332	ESTs, highly similar to growth factor-responsive protein, vascular smooth muscle [*R. norvegicus*]	41	14.1	96.4	96.4	hCG41109	2×10^{-62}
3	T72235	Nicotinamide N-methyltransferase	42	13.5	96.6	96.6	hCG39357	10^{-102}
4	W72051	Fatty acid binding protein 7, brain	43	13.2	87.5	75.0	hCG19286	10^{-180}
5	W70343	Lysyl oxidase	44	11.2	95.8	87.5	hCG37363	10^{-75}
6	H99075	ESTs	44	10.7	95.7	87.0	hCG37363	0
7	W30988	ESTs, highly similar to angiopoietin-related protein [*H. sapiens*]	45	11.1	100.0	100.0	hCG23958	10^{-111}
8	T54298		45	8.1	100.0	96.6	hCG23958	10^{-153}
9	N50654	Ceruloplasmin (ferroxidase)	46	10.6	95.8	95.8	hCG21214	10^{-146}
10	W93163	Tumor necrosis factor, α-induced protein 6	47	10.5	100.0	100.0	hCG41965	6×10^{-76}
11	AA598601	Insulin-like growth factor binding protein 3	48	7.6	96.6	89.7	hCG18013	0
12	AA678335	*H. sapiens* phosphodiesterase I/nucleotide yrophosphatase 3 (PDNP3) mRNA	49	7.6	84.0	84.0	hCG18059	10^{-145}
13	AA164819	ESTs	50	7.1	96.3	88.9	hCG38036	0
14	AA485896		50	6.8	96.4	89.3	hCG38036	10^{-145}
15	N26171	ESTs	51	6.2	87.5	79.2	hCG19701	0
16	AA487787	Von Willebrand factor	52	6.2	100.0	87.5	hCG24322	10^{-168}
17	AA450189	Enolase 2, (γ, neuronal)	53	6.0	96.4	92.9	hCG25937	0
18	R62612	Fibronectin 1	54	5.6	93.1	79.3	hCG16692	2×10^{-51}
19	H20872	FcγIIIaR (CD16); low affinity receptor for IgG Fc fragment	55	5.5	85.7	82.1	hCG16608	0
20	W72293	ESTs	56	5.5	93.1	89.7	hCG20029	2×10^{-68}
21	AA055835	Caveolin 1, caveolae protein, 22kD	57	5.4	92.9	75.0	hCG39088	10^{-121}
22	AA873159	Apolipoprotein C-I	58	5.3	88.9	81.5	hCG22139	4×10^{-68}
23	AA017544	Regulator of G-protein signalling 1	59	5.2	85.7	82.1	hCG39901	10^{-179}
24	R19956	Vascular endothelial growth factor	60	5.1	96.4	85.7	hCG18998	4×10^{-90}
25	H99816	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase) 2	61	5.1	96.4	78.6	hCG16089	10^{-149}
26	R49597	ESTs	62	4.6	95.8	75.0	hCG15938	4×10^{-83}
27	AA405000	*H. sapiens* ribonuclease 6 precursor mRNA	63	4.5	96.2	80.8	hCG15018	10^{-149}

28	H58873	Solute carrier family 2 (facilitated glucose transporter), member 1	64	4.5	93.1	86.2	hCG23157	10^{-176}
29	T62491	Chemokine (C-X-C motif), receptor 4 (fusin)	65	4.4	89.7	75.9	hCG25754	10^{-128}
30	AA443899	CD36 (collagen type I receptor, thrombospondin receptor-like 1	66	4.2	89.3	75.0	hCG25301	0
31	AI004331	Human MHC class II HLA-DQβ mRNA (DR7 DQw2), complete cds	67	4.1	85.7	78.6	CG201516	0
32	AA488892	ESTs, Weakly similar to gag-pol polyprotein [*M. musculus*]	68	4.0	85.7	75.0	hCG95780	5×10^{-89}

* The values in this column are the % of CC-RCC patients in whom a given gene was expressed at least 3-fold higher compared to control kidney tissue. Genes included in this Table met or exceeded this threshold in at least 75% of CC-RCC patients.
** The values in this column are the % of CC-RCC patients in whom a given gene was expressed at least 2-fold higher compared to control kidney tissue. Genes included in this Table met or exceeded this threshold in at least 75% of CC-RCC patients.

Table 3. Second Set of Commonly Up-Regulated Genes in CC-RCC

	GenBank Accession #	Name (from Research Genetics database)	SEQ ID NO:	Average Fold Up	Incidence % of RCC*
1	AA101875	Chondroitin sulfate proteoglycan 2 (versican)	140	5.5	80.8
2	W60845	Cell division cycle 42 (GTP-binding protein, 25kD)	141	4.8	77.8
3	AA457700	Cytochrome b-561	142	4.8	88.9
4	H95819	ESTs	143	4.7	91.3
5	AA136707	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase) 2	144	4.5	96.4
6	R43605	KIAA0293 protein	145	4.4	93.1
7	N63943	Lysozyme (renal amyloidosis)	146	4.2	82.8
8	N76878	Decidual protein induced by progesterone	147	4.1	86.2
9	R95749	ESTs	148	4.1	88.9
10	AA417622	ESTs	149	4.0	92.9
11	AA460224	ESTs	150	3.9	92.6
12	AA460152	Serum-inducible kinase	151	3.9	86.2
13	W72329	Lymphotoxin α (TNF superfamily, member 1)	152	3.9	82.1
14	AA700054	Adipose differentiation-related protein; adipophilin	153	3.9	86.2
15	W80701	ESTs, Weakly similar to HERV-E envelope glycoprotein [H.sapiens]	154	3.8	91.7
16	AA442984	major histocompatibility complex (MHC) class II, DQβ1	155	3.8	86.2
17	H12338	TYRO protein tyrosine kinase binding protein	156	3.8	89.7
18	AA629189	keratin 4	157	3.8	85.7

19	AA176581	myoglobin	158	3.8	82.1
20	R33363	decidual protein induced by progesterone	159	3.7	93.1
21	AA456821	ESTs, Weakly similar to intrinsic factor-B12 receptor precursor [H.sapiens]	160	3.7	87.0
22	R43734	laminin, α4	161	3.7	82.1
23	N38801	ESTs, Highly similar to Complement C1q subcomponent, C chain precursor	162	3.7	89.7
24	AA458472	MHC, class II, DQ β 1	163	3.6	79.3
25	AA489611	lactate dehydrogenase A	164	3.5	89.7
26	N90491	ESTs, Highly similar to Complement C1q subcomponent, C chain precursor	165	3.5	88.9
27	N30205	ESTs	166	3.4	79.3
28	R47979	Human HLA-DR α-chain mRNA	167	3.4	75.9
29	T62849	ESTs	168	3.4	81.8
30	AA425450	glycoprotein (transmembrane) nmb	169	3.4	81.5
31	N94616	laminin, α 4	170	3.3	77.8
32	AA478542	A kinase (PRKA) anchor protein (gravin) 12	171	3.3	79.3
33	AA236164	cathepsin S	172	3.3	85.7
34	AA677340	phosphorylase kinase, α 2 (liver)	173	3.3	82.1
35	AA002126	apoptosis inhibitor 2	174	3.3	84.0
36	AA486627	MHC class II, DPβ1	175	3.3	75.9
37	AA486567	ESTs	176	3.3	91.7
38	W37864	phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	177	3.3	88.9
39	H15662	KIAA0291 protein	178	3.3	80.8
40	N71028	ESTs	179	3.3	82.8
41	AA421296	CD68 antigen	180	3.3	82.8
42	W60701	MHC class I, A	181	3.2	75.9
43	AA599138	ESTs	182	3.2	89.3
44	AA634028	Human mRNA for SB class II MHC α-chain	183	3.2	79.3
45	AA682558	ESTs	184	3.2	81.8
46	AA132090	CD53 antigen	185	3.2	81.5
47	R97251	Homo sapiens clone 24655 mRNA sequence	186	3.1	85.7
48	H79353	Fc fragment of IgE, high affinity I, receptor for; γ polypeptide	187	3.1	85.7
49	W73144	lymphocyte cytosolic protein 1 (L-plastin)	188	3.1	79.3
50	AI005515	hexokinase 2	189	3.1	81.8
51	AA478585	butyrophilin, subfamily 3, member A3	190	3.0	75.9
52	AA126982	sin3-associated polypeptide, 30kD	191	3.0	80.0
53	AA644657	MHC class I, A	192	3.0	82.8

54	AA425806	Suppressin (nuclear deformed epidermal autoregulatory factor-1 (DEAF-1)-related)	193	3.0	78.6
55	T89391	Caveolin 2	194	3.0	78.6
56	H11732	ESTs	195	3.0	79.2
57	AA083407	Stimulated trans-acting factor (50 kDa)	196	3.0	77.8
58	AA702254	MHC, class II, DN α	197	2.9	82.8
59	W88967	MHC, class II, DR β 1	198	2.9	75.9
60	AA491191	interferon, γ-inducible protein 16	199	2.9	82.1
61	AA157813	interferon, α-inducible protein 27	200	2.9	76.9
62	AA777488	ESTs, moderately similar to glyceraldehyde-3-phosphate dehydrogenase, liver	201	2.9	75.9
63	N66053	Butyrophilin, subfamily 3, member A1	202	2.9	75.9
64	AA988615	Human HLA-F gene	203	2.8	78.6
65	N32226	ESTs	204	2.8	77.8
66	T63324	MHC, class II, DQ α1	205	2.8	75.9
67	AA669055	MHC, class II, DQ β1	206	2.8	79.3
68	AA102068	heat shock transcription factor 4	207	2.8	78.6
69	H95960	secreted protein, acidic, cysteine-rich (osteonectin)	208	2.8	75.9
70	T84762	Homo sapiens mRNA; cDNA DKFZp434O071 (from clone DKFZp434O071)	209	2.8	85.2
71	AA664195	MHC, class II, DR β1	210	2.8	75.0
72	AA453978	GM2 ganglioside activator protein	211	2.7	84.0
73	AA464246	MHC, class I, C	212	2.7	82.8
74	H26176	Homo sapiens mRNA; cDNA DKFZp564E1616 (from clone DKFZp564E1616)	213	2.7	77.8
75	AA456063	Homo sapiens mRNA for hCRNN4, complete cds	214	2.7	78.6
76	AA708621	ESTs, [H.sapiens]	215	2.7	89.3
77	AA284954	colony stimulating factor 1 receptor, formerly McDonough feline sarcoma viral (v-fms) oncogene homolog	216	2.7	85.2
78	AA521292	ATP-binding cassette, sub-family A (ABC1), member 1	217	2.7	80.8
79	R92609	colony stimulating factor 1 receptor, formerly McDonough feline sarcoma viral (v-fms) oncogene homolog	218	2.6	77.3
80	R33609	ESTs	219	2.6	82.8
81	AA430540	collagen, type IV, α 2	220	2.6	79.3
82	H41165	Ribosomal protein S19	221	2.6	82.8
83	AA485371	Bone marrow stromal cell antigen 2	222	2.5	75.9
84	AA461309	ESTs, Weakly similar to predicted using Genefinder [C.elegans]	223	2.5	75.0
85	AA130874	ESTs, Weakly similar to [H.sapiens]	224	2.5	75.9
86	T62048	Complement component 1, s subcomponent	225	2.5	76.0

87	T69304	TAP binding protein (tapasin)	226	2.5	79.2
88	AA463188	Putative serine-threonine protein kinase	227	2.5	81.5
89	W37721	ESTs	228	2.4	75.9
90	AA862434	Proteasome (macropain) subunit, β type, 9 (large multifunctional protease 2)	229	2.4	75.0
91	R22412	Platelet/endothelial cell adhesion molecule (CD31 antigen)	230	2.4	75.9

*The values in this column are the % of CC-RCC patients in whom a given gene was expressed at least 2-fold higher compared to control kidney tissue. Genes included in this Table met or exceeded this threshold in at least 75% of CC-RCC patients (but did not exceed the threshold of 3 fold upregulation in this percentage of patients

Table 4. First Set of Commonly Down-Regulated Genes in CC-RCC

	GenBank Accession #	Name (from Research Genetics database)	SEQ ID NO:	Averag e fold Down	Incidence % of RCC*		Celera No#	E value§
					2-fold *	3-fold**		
1	R89067	Kininogen	69	27.2	100.0	100.0	hCG16151	10^{-161}
2	AA705692	ESTs	69	18.0	100.0	100.0	hCG16151	0
3	T53220	Fatty acid binding protein 1, liver	70	22.8	95.8	95.8	hCG32947	10^{-49}
4	AA682293	Phenylalanine hydroxylase	71	20.4	96.0	96.0	hCG21871	0
5	AA954947	Epidermal growth factor (β-urogastrone)	72	15.0	100.0	100.0	hCG19911	0
6	H72098	aldolase B, fructose-bisphosphate	73	13.6	100.0	96.6	hCG27655	0
7	AA411988	EST	74	13.3	100.0	96.4	hCG28257	0.014
8	T73187	Plasminogen	75	12.0	100.0	89.3	hCG32944	3×10^{-56}
9	T51617	solute carrier family 22 (organic cation transporter, member 3)	75	11.8	96.4	92.9	hCG32944	10^{-169}
10	AA777384	ESTs	76	11.0	96.2	92.3	hCG17572	0
11	H53340	Metallothionein 1G	77	10.0	100.0	93.1	hCG40931	7×10^{-64}
12	AA844930	Glycoprotein 2 (zymogen granule membrane)	78	9.6	100.0	96.6	hCG34445	2×10^{-89}
13			79	9.4	96.6	96.6		
14	AA858026	protein C inhibitor -plasminogen activator inhibitor 3	80	9.4	100.0	96.6	hCG40087	4×10^{-4}
15	H18950	ESTs, similar to hepatocyte nuclear factor 4 γ [*H. sapiens*]	81	9.2	100.0	96.6	hCG40025	10^{-169}
16			82	8.9	96.6	96.6		
17	AA040387	X-prolyl aminopeptidase (aminopeptidase P) 2, membrane-bound	83	8.8	96.4	96.4	hCG20708	10^{-133}
18	H77766	Metallothionein 1H	84	8.4	96.6	86.2	hCG23909	10^{-151}
19	N55459	RNA helicase-related protein	84	6.9	96.6	79.3	hCG23909	6×10^{-97}
20	H72722	ESTs, similar to metallothionein-IB [*H. sapiens*]	84	5.2	86.2	75.9	hCG23909	10^{-61}

21	W16424	ESTs	85	8.4	92.6	85.2	hCG41126	0
22	H88329	Calbindin 1, (28kD)	86	8.1	100.0	92.9	hCG33059	10^{-148}
23	N62179	Methylmalonate-semialdehyde dehydrogenase	87	7.9	100.0	96.4	hCG21723	10^{-179}
24	AA460298		87	4.4	91.7	75.0	hCG21723	0
25	AA775872	Glypican 3	88	7.9	100.0	100.0	hCG14619	10^{-133}
26	AA457718	*H. sapiens* mRNA; cDNA DKFZp564B076 (from clone DKFZp564B076)	89	7.8	95.7	87.0	hCG18130	0
27	R24266	Growth factor receptor-bound protein 14	90	7.1	80.8	76.9	hCG40120	10^{-104}
28	R54778	Collagen, type XVI, α1	91	7.1	100.0	95.8	hCG41613	10^{-101}
29	AA702640	DOPA decarboxylase (aromatic L-amino acid decarboxylase)	92	7.0	96.3	85.2	hCG18339	0
30	AA664180	Glutathione peroxidase 3 (plasma)	93	6.6	92.9	85.7	hCG39155	2×10^{-74}
31	R10382	Protein C inhibitor (plasminogen activator inhibitor 3 (PAI-3)	94	6.4	92.6	88.9	hCG16021	0.058
32	AA227594	Mal, T-cell differentiation protein	95	6.3	100.0	86.2	hCG38742	10^{-141}
33	H68509	UDP glycosyltransferase-2 family, polypeptide B10	96	6.1	95.5	86.4	hCG41481	0.25
34	AA676466	Argininosuccinate synthetase	97	6.1	96.4	85.7	hCG40893	0
35	H96140	acyl-coenzyme A dehydrogenase, short/branched chain	98	6.0	96.0	96.0	hCG40572	10^{-18}
36	H11346	Aldehyde dehydrogenase 4 (glutamate γ-semialdehyde dehydrogenase; pyrroline-5-carboxylate dehydrogenase)	99	6.0	92.9	89.3	hCG25108	0
37	AA862999	calcium-sensing receptor (hypercalcemia 1, severe neonatal hyperparathyroidism)	100	6.0	100.0	92.9	hCG14928	0
38	AA497001	ESTs, weakly similar to BcDNA.GH02901 [*D. melanogaster*]	101	6.0	96.3	88.9	hCG29639	0
39	AA449780	EST	102	5.9	88.9	77.8	hCG32613	2×10^{-36}
40	H11369	aldehyde dehydrogenase 4 (glutamate γ-semialdehyde dehydrogenase; pyrroline-5-carboxylate dehydrogenase)	103	5.8	92.9	85.7	hCG37443	10^{-151}
41	AA704995	Putative glycine-N-acyltransferase	104	5.6	92.9	78.6	hCG38673	0
42	T94781	Potassium inwardly-rectifying channel, subfamily J, member 15	105	5.6	92.9	78.6	hCG22477	0.07
43	N89673	ESTs	106	5.6	92.6	81.5	hCG39647	5×10^{-59}
44	H37880	ESTs,	107	5.6	96.3	88.9	hCG29091	0
45	AA663884	synaptosomal-associated protein, 25kD	108	5.5	95.7	87.0	hCG40236	3×10^{-88}
46	R25818	aldehyde dehydrogenase 9 (γ-aminobutyraldehyde dehydrogenase, E3 isozyme)	109	5.5	100.0	91.3	hCG21745	10^{-142}
47	AA700604	Sorbitol dehydrogenase	110	5.4	92.6	85.2	hCG96145	0.36
48	W95082	Hydroxysteroid (11-β) dehydrogenase 2	111	5.4	96.6	89.7	hCG27201	10^{-132}
49	AA677655	Klotho	112	5.4	92.3	80.8	hCG32197	0
50	N80129	metallothionein 1L	113	5.3	86.2	79.3	hCG24714	10^{-111}
51	AA402915	aminoacylase 1	114	5.3	96.3	85.2	hCG42576	4×10^{-98}
52	AA863424	dipeptidase 1 (renal)	115	5.2	93.1	82.8	hCG18560	0.05

53	N78083	Glycine dehydrogenase (decarboxylating; glycine decarboxylase, glycine cleavage system protein P)	116	5.1	96.4	92.9	hCG31017	9×10^{-69}
54	R06601	ESTs, Moderately similar to metallothionein-II [*H. sapiens*]	117	5.1	82.8	75.9	hCG39693	4×10^{-34}
55	AA872383	Metallothionein 1E (functional)	117	4.8	82.8	75.9	hCG39693	10^{-86}
56	AA131240	ESTs	118	5.0	92.0	80.0	hCG14827	0.02
57	AA485965	Succinate-CoA ligase, GDP-forming, α subunit	119	4.9	92.9	89.3	hCG33938	10^{-114}
58	AA196287	ESTs, weakly similar to alternatively spliced product using exon 13A [*H. sapiens*]	120	4.9	96.6	89.7	hCG21724	0
59	R61229	glycine amidinotransferase (L-arginine:glycine amidinotransferase)	121	4.8	82.8	75.9	hCG38743	10^{-138}
60	N23898	G protein-coupled receptor kinase 2 (Drosophila-like)	122	4.8	92.9	82.1	hCG20632	4×10^{-89}
61	AA699427	Fructose-bisphosphatase 1	123	4.7	93.1	82.8	hCG32887	0
62			124	4.7	96.2	84.6		
63	AA873355	ATPase, Na+/K+ transporting, α 1 polypeptide	125	4.7	100.0	93.1	hCG37943	10^{-105}
64	AI000188	UDP glycosyltransferase 2 family, polypeptide B7	126	4.6	85.7	75.0	hCG40932	2×10^{-61}
65	N53031	UDP glycosyltransferase 2 family, polypeptide B4	126	4.0	86.2	75.9	hCG40932	2×10^{-12}
66	AA459197	Sodium channel, nonvoltage-gated 1 α	127	4.6	89.7	75.9	hCG24314	0
67	W86431	Protein C inhibitor (PAI-3)	128	4.4	100.0	76.9	hCG22335	8×10^{-5}
68	T65482	L-3-hydroxyacyl-Coenzyme A dehydrogenase, short chain	129	4.4	96.2	76.9	hCG19900	10^{-127}
69	AA457374	DKFZP586B0319 protein	130	4.3	91.7	75.0	hCG20212	0
70	R33037	ESTs	131	4.3	92.0	76.0	hCG18871	10^{-140}
71	AA437099	ESTs	132	4.3	85.2	77.8	hCG18095	0
72	W01011	SA (rat hypertension-associated) homolog	133	4.2	89.7	75.9	hCG37242	4×10^{-84}
73	R16596	EST, moderately similar to Cd-7 Metallothionein-2 [*H. sapiens*]	134	4.1	86.2	75.9	hCG21792	2×10^{-67}
74	AA863449	Oviductal glycoprotein 1, 120kD	135	4.0	92.9	75.0	HCG39984	10^{-141}
75	AA458884	S100 calcium-binding protein A2	136	4.0	92.9	75.0	hCG15472	6×10^{-90}
76	AA608575	Propionyl coenzyme A carboxylase, α polypeptide	137	3.8	89.7	75.9	hCG24579	10^{-116}
77	H18608	Solute carrier family 22 (organic anion transporter), member 8	138	3.6	89.3	78.6	hCG21316	10^{-141}

* The values in this column are the % of CC-RCC patients in whom a given gene was expressed at least 3-fold lower compared to control kidney tissue. Genes included in this Table met or exceeded this threshold in at least 75% of CC-RCC patients.

** The values in this column are the % of CC-RCC patients in whom a given gene was expressed at least 2-fold lower compared to control kidney tissue. Genes included in this Table met or exceeded this threshold in at least 75% of CC-RCC patients.

§ The E Value is a statistical value reflecting the probability that the match between the probe sequence and the sequence in the Celera database is due to chance alone. Thus very low values indicate virtual certainty that the sequence being queried corresponds to the particular gene in the database.

Table 5. Second Set Commonly Down-Regulated Genes in CC-RCC

	GenBank Accession #	Name (from Research Genetics database)	SEQ ID NO:	Average Fold Down	Incidence % of RCC*
1	AA454810	Membrane component, chrom. 1, surface marker 1 (40kD glycoprotein, identified by monoclonal antibody GA733)	231	5.2	88.5
2	N73241	Solute carrier family 17 (sodium phosphate), member 1	232	5.2	91.7
3	W85851	ESTs	233	5.2	75.9
4	AA047666	Flavin containing monooxygenase 1	234	4.9	82.1
5	AA455632	Human chromosome 3p21.1 gene sequence, complete cds	235	4.8	89.3
6	R98851	Membrane metallo-endopeptidase (neutral endopeptidase, enkephalinase, CALLA, CD10)	236	4.6	82.8
7	N74679	Homo sapiens mRNA for G3a protein (located in the class III region of the MHC	237	4.6	85.2
8	N51498	ESTs	238	4.6	82.6
9	R60170	ESTs	239	4.5	75.0
10	AA460012	Solute carrier family 22 (organic cation transporter), member 3	240	4.4	95.7
11	R42433	H.sapiens mRNA for protein tyrosine phosphatase	241	4.4	87.0
12	R83190	ESTs, similar to alanine-glyoxylate aminotransferase 2 precursor from rat (*R. norvegicus*)	242	4.3	88.5
13	R97050	ESTs	243	4.2	85.7
14	T67549	Plasminogen	244	4.2	86.4
15	AA862465	Alpha-2-glycoprotein 1, zinc	245	4.1	93.1
16	H08720	ESTs	246	4.1	96.4
17	N35592	ESTs	247	4.1	93.1
18	AA284067	ESTs	248	4.0	92.6
19	AA447115	Stromal cell-derived factor 1	249	4.0	79.2
20	T50788	UDP glycosyltransferase 2 family, polypeptide B15	250	4.0	85.2
21	AA448710	ESTs	251	4.0	77.8
22	AA480851	Claudin 10	252	3.8	89.3
23	AA099593	KIAA0977 protein	253	3.8	88.9
24	T58958	Betaine-homocysteine methyltransferase	254	3.8	79.3
25	AA872602	Parathyroid hormone receptor 1	255	3.8	93.1
26	R44346	ESTs, Weakly similar to T27A1.5 [*C. elegans*]	256	3.8	86.2
27	AA452278	Solute carrier family 4, sodium bicarbonate cotransporter, member 4	257	3.7	85.2
28	N74025	Homo sapiens deiodinase, iodothyronine, type I (DIO1) mRNA	258	3.7	92.3
29	AA757672	ESTs	259	3.6	100.0
30	AA135958	ESTs	260	3.6	75.9

31	AA455800	Gamma-glutamyl hydrolase (conjugase, folylpoly-γ-glutamyl hydrolase)	261	3.6	82.1
32	AA504160	ATPase, H+ transporting, lysosomal (vacuolar proton pump), α polypeptide, 70kD, isoform 1	262	3.6	95.5
33	AA044205	ESTs	263	3.6	80.0
34	H90507	Plasminogen	264	3.5	82.1
35	AA427619	ESTs, Weakly similar to α 1,2-mannosidase IB [H.sapiens]	265	3.5	91.7
36	W02265	Translational inhibitor protein p14.5	266	3.5	75.9
37	H17921	ESTs	267	3.5	92.0
38	R76505	ESTs	268	3.5	96.4
39	AA476258	ESTs	269	3.5	87.5
40	N91990	phytanoyl-CoA hydroxylase (Refsum disease)	270	3.5	88.0
41	T98253	ESTs	271	3.5	93.1
42	W84868	cytochrome P450, subfamily IVA, polypeptide 11	272	3.4	84.6
43	H05140	regucalcin (senescence marker protein-30)	273	3.4	85.7
44	H09818	ESTs	274	3.4	82.6
45	W01048	ESTs	275	3.3	77.8
46	AI289110	metallothionein 1L	276	3.3	82.8
47	H77535	ESTs, Weakly similar to choline kinase isolog 384D8_3 [H.sapiens]	277	3.3	83.3
48	T60160	ESTs, Moderately similar to MM46 [H.sapiens]	278	3.3	89.7
49	H14604	ESTs, Weakly similar to *C. elegans* cDNA yk30b3.5	279	3.3	79.2
50	T98394	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 7	280	3.3	85.7
51	H48148	ESTs, Weakly similar to AIF-1 [H.sapiens]	281	3.3	79.3
52	AA453783	Homo sapiens mRNA; cDNA DKFZp564B1264 (from clone DKFZp564B1264)	282	3.3	82.1
53	T69767	hydroxyacyl-coenzyme A dehydrogenase/3-ketoacyl-coenzyme A thiolase/enoyl-coenzyme A hydratase (trifunctional protein), β subunit	283	3.2	84.0
54	AA446650	Homo sapiens mRNA; cDNA DKFZp586M0723 (from clone DKFZp586M0723)	284	3.2	75.0
55	AA416875	ESTs	285	3.2	95.7
56	R54850	biphenyl hydrolase-like (serine hydrolase; breast epithelial mucin-associated antigen)	286	3.2	85.7
57	AA775957	ATPase, Na+/K+ transporting, α 3 polypeptide	287	3.2	93.1
58	AA455222	plasminogen activator, urokinase receptor	288	3.2	77.8
59	AA406266	ESTs	289	3.1	84.6
60	N26658	ESTs, Moderately similar to TGF-β Receptor type III precursor [H.sapiens]	290	3.1	82.1
61	AA455969	prion protein (p27-30) (Creutzfeld-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia)	291	3.1	89.7
62	N70794	acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain	292	3.1	79.3
63	AA463454	ESTs, [*H.sapiens*]	293	3.1	82.1

64	H23187	carbonic anhydrase II	294	3.0	79.3
65	AA205598	ESTs	295	3.0	89.3
66	AA670438	ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)	296	3.0	75.9
67	AA121668	pigment epithelium-derived factor	297	3.0	86.2
68	AA487346	cathepsin H	298	3.0	92.0
69	W84701	solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	299	2.9	77.8
70	AA456022	ESTs, weakly similar to unknown [H.sapiens]	300	2.9	81.5
71	AA424905	ESTs	301	2.9	86.2
72	W87747	ESTs	302	2.9	78.3
73	R76614	ESTs	303	2.9	78.3
74	AA132964	ESTs	304	2.9	78.6
75	AA169798	biphenyl hydrolase-like (serine hydrolase; breast epithelial mucin-associated antigen)	305	2.8	86.2
76	H15504	Annexin A7	306	2.8	85.2
77	H22856	Glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1)	307	2.8	82.1
78	AA429946	ESTs, Highly similar to peroxisomal short-chain alcohol dehydrogenase [H.sapiens]	308	2.8	82.8
79	R93551	aldehyde dehydrogenase 5	309	2.8	85.7
80	AA256123	fragile histidine triad gene	310	2.8	81.8
81	AA621183	solute carrier family 5 (inositol transporters), member 3	311	2.8	76.0
82	R67147	crystallin, μ	312	2.8	76.0
83	AA156988	iron-responsive element binding protein 1	313	2.7	78.6
84	AA459668	3-hydroxyisobutyryl-coenzyme A hydrolase	314	2.7	75.9
85	N46098	biphenyl hydrolase-like (serine hydrolase; breast epithelial mucin-associated antigen)	315	2.6	77.8
86	W81371	ESTs	316	2.6	75.9
87	H99883	KIAA0828 protein	317	2.6	79.2
88	N65985	ESTs	318	2.6	75.9
89	AA400258	Human DNA sequence from clone 215D11, chromosome 1p36 12-36.33 Contains a gene for a RNA-binding protein regulatory subunit, gene similar to rat gene 33, pseudogene similar to PLA-X, ESTs, STSs, GSSs and CpG islands	319	2.5	75.0
90	AA456595	ESTs	320	2.5	78.3
91	N31492	flavin containing monooxygenase 4	321	2.5	75.0
92	R28294	glycine cleavage system protein H (aminomethyl carrier)	322	2.5	85.7
93	AA430382	nucleoside phosphorylase	323	2.5	82.8
94	AA521401	pyruvate dehydrogenase (lipoamide) β	324	2.4	78.6
95	AA453691	Aminolevulinate, delta-, synthase 1	325	2.4	75.9
96	N99256	ESTs	326	2.4	80.0
97	AA448184	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	327	2.4	75.9

98	AA056390	RD RNA-binding protein	328	2.4	81.5
99	H78368	ESTs	329	2.3	76.9
100	AA432268	ESTs	330	2.3	75.9
101	AA453679	Dihydrolipoamide dehydrogenase (E3 component of pyruvate dehydrogenase complex, 2-oxo-glutarate complex, branched chain keto acid dehydrogenase complex)	331	2.3	75.9

*The values in this column are the % of CC-RCC patients in whom a given gene was expressed at least 2-fold lower compared to control kidney tissue. Genes included in this Table met or exceeded this threshold in at least 75% of CC-RCC patients (but did not exceed the threshold of 3 fold down-regulation in this percentage of patients

Table 6A and 6B: First Set of Genes Differentially Expressed in Aggressive vs. Non-aggressive type CC-RCC

	GenBank Accession #	NAME (from Research Genetics)	SEQ ID NO:	Celera #	E value
		Table 6A Genes upregulated in non aggressive CC-RCC			
1	N35086	FYN oncogene related to *Src, Fgr, Yes*	1	hCG34806	0
2	N66144		1	hCG34806	10^{-153}
3	T47312	Insulin receptor	2	hCG21793	10^{-180}
4	AA001614		2	hCG21793	10^{-114}
5	T80232	phosphodiesterase I/nucleotide pyrophosphatase 2 (autotaxin)	3	hCG21270	10^{-100}
6	AA490694	Hevin	4	hCG38543	4×10^{-78}
7			5		
8	AA486082	serum/glucocorticoid regulated kinase	6	hCG32737	8×10^{-99}
9	W60845	cell division cycle 42 (GTP-binding protein)	7	hCG15193	10^{-138}
10	N34362	regulator of G-protein signalling 5	7	hCG15193	0
11	AA668470		7	hCG15193	0
12	H84815	Rab9 effector p40	8	hCG29658	10^{-155}
13	AA669136	transcription factor 4	9	hCG22018	10^{-127}
14	N39240	ESTs	9	hCG22018	0
15	W72803	ESTs, weakly similar to KIAA0768 protein	10	hCG28803	0.081
16	R22412	platelet/endothelial cell adhesion molecule	11	hCG40093	10^{-12}
17	R56211	platelet-derived growth factor receptor, β polypeptide	12	hCG16146	0.99
18	H74106	LIM binding domain 2	13	hCG40704	0
19	H72113	CD34 antigen	14	hCG21280	10^{-153}
20	AA680300	*H. sapiens* clone 23698 mRNA			
21	R32440		15	hCG17031	10^{-112}
22	AA777910	*H. sapiens* clone 23698 mRNA	139	no list	

23			16		
24	AA432292	ESTs	17	hCG29296	0.26
25	AA055440	Sprouty (Drosophila) homolog 1, antagonist of FGF signal	18	hCG28465	0.012
26	H68922	Integrin, α1	19	hCG23896	0.08
27	N29914	Endothelin receptor type B	20	hCG32240	10^{-111}
28			21		
	GENBANK ACCESSION #	NAME (FROM RESEARCH GENETICS)	SEQ ID NO:	CELERA #	E VALUE

Table 6B Genes downregulated in aggressive CC-RCC

29	AA775447	α-2-macroglobulin	22	hCG25215	7×10^{-67}
30	H99415	a kinase (PRKA) anchor protein 2	23	hCG28766	10^{-158}
31	N51499		23	hCG28766	0
32	AA464644	LIM domain only 2 (rhombotin-like 1)	24	hCG26502	0
33	N74956	DNA-directed RNA polymerase II B (140 kD)	25	hCG201171	3×10^{-45}
34	T53298	insulin-like growth factor binding protein 7	25	hCG201171	5×10^{-15}
35	AA704965	ESTs	26	hCG41053	0.018
36	AA099153	**tissue inhibitor of metalloproteinase 3 (TIMP)**	27	hCG41415	10^{-174}
37	N95226	KIAA0758	28	hCG18763	0
38	T63971		28	hCG18763	10^{-121}
39	AA189106	KIAA1102	29	hCG33090	0
40	R23270		29	hCG33090	10^{-104}
41	N36136	ESTs, moderately similar to endomucin	30	hCG39439	0
42	N93505	Transmembrane 4 superfamily member 2	31	hCG18324	10^{-160}
43	AA173408	ESTs	32	hCG37431	10^{-109}
44	T71976	Phosphatidic acid phosphatase type 2b	33	hCG32470	0
45	T72119		33	hCG32470	10^{-154}
46	AA487034	**Transforming growth factor β receptor II (70-80kD)**	34	hCG26855	10^{-127}
47	W68396	KIAA0096	35	hCG26802	10^{-127}
48	N57594	*H. sapiens* mRNA; cDNA DKFZp564E153	36	hCG41872	0.98
49	N94344	*H. sapiens* mRNA; cDNA DKFZp564E153	37	hCG15077	0.005
50	W47641	*H. sapiens* mRNA; cDNA DKFZp564E153	38	hCG25175	1.4
51	AA458653	*H. sapiens* mRNA for GS3955, complete cds	39	hCG15902	2×10^{-94}

Table 7. Second Set of Genes (166) Differentially Expressed in Aggressive vs. Non-aggressive type CC-RCC

	GenBank Accession #	Name	SEQ ID NO:
1	H23081	Zinc finger protein 264	332
2	N25425	v-raf-1 murine leukemia viral oncogene homologue 1	333
3	AA670438	Ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)	334
4	AA453273	U6 snRNA-associated Sm-like protein	335
5	AA405748	U2 small nuclear ribonucleoprotein auxiliary factor (65kD)	336
6	AA432062	Tyrosine kinase with immunoglobulin and epidermal growth factor homology domains	337
7	R06309	Tumor protein D52-like 2	338
8	R36467	Transforming growth factor, β 1	339
9	H50377	Tight junction protein 1 (zona occludens 1)	340
10	AA778098	Thymidine kinase 1, soluble	341
11	H05577	Splicing factor 30, survival of motor neuron-related	342
12	AA018591	Spectrin, β, non-erythrocytic 1	343
13	R66139	Small inducible cytokine subfamily D (Cys-X3-Cys), member 1 (fractalkine, neurotactin)	344
14	R96668	Small inducible cytokine subfamily A (Cys-Cys), member 14	345
15	N64837	SFRS protein kinase 1	346
16	AA070226	Selenoprotein P, plasma, 1	347
17	W96107	Sec61 γ	348
18	R55105	Sarcoglycan, β (43kD dystrophin-associated glycoprotein)"	349
19	N93715	Ribosomal protein S29	350
20	AA991856	Ribophorin II	351
21	AA479781	Radixin	352
22	AA464152	Quiescin Q6	353
23	AA151249	Protoporphyrinogen oxidase	354
24	R79082	protein tyrosine phosphatase, receptor type, K	355
25	AI022531	protein tyrosine phosphatase, receptor type, β polypeptide	356
26	AA490696	protein phosphatase 2 (formerly 2A), catalytic subunit, β isoform	357
27	AA916327	protective protein for β-galactosidase (galactosialidosis)	358
28	AA455193	proteasome (prosome, macropain) 26S subunit, non-ATPase, 2	359
29	AA426212	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), β polypeptide (protein disulfide isomerase; thyroid hormone binding protein p55)	360
30	AA488432	phosphoserine phosphatase	361
31	AA402874	phospholipid transfer protein	362

32	AA699876	phosphoinositide-3-kinase, class 2, β polypeptide	363
33	AA629987	peptidylprolyl isomerase D (cyclophilin D)	364
34	AA488969	PDZ domain containing guanine nucleotide exchange factor(GEF)1; RA(Ras/Rap1A-associating)-GEF	365
35	AA845432	parathyroid hormone-like hormone	366
36	AA446301	paraoxonase 2	367
37	AA451781	novel RGD-containing protein	368
38	H83225	Novel gene on chromosome 20	369
39	AA709414	nidogen (enactin)	370
40	N30706	neuralized (Drosophila)-like	371
41	AA491124	NAD(P)H menadione oxidoreductase 2, dioxin-inducible	372
42	R44617	MyoD family inhibitor	373
43	R59167	Meis (mouse) homolog 2	374
44	AA155913	matrix Gla protein	375
45	R39273	MAD (mothers against decapentaplegic, Drosophila) homolog 4	376
46	AA668531	leucocyte vacuolar protein sorting 45	377
47	AA459106	kinectin 1 (kinesin receptor)	378
48	N70078	KIAA1058 protein	379
49	H60026	KIAA0745 protein	380
50	AA455507	KIAA0618 gene product	381
51	AA702698	KIAA0414 protein	382
52	AA284634	Janus kinase 1 (a protein tyrosine kinase)	383
53	R70685	Jagged1 (Alagille syndrome)	384
54	AA683550	interleukin-1 receptor-associated kinase 1	385
55	AA148200	integrin-linked kinase	386
56	N74131	Human secretory protein (P1.B) mRNA, complete cds"	387
57	AA487681	Human mRNA for ornithine decarboxylase antizyme, ORF 1 and ORF 2	388
58	AA931758	Human G0S2 protein gene, complete cds	389
59	AA418914	Human DNA sequence from clone 30M3 on chromosome 6p22.1-22.3. Contains three novel genes, one similar to C. elegans Y63D3A.4 and one similar to (predicted) plant, worm, yeast and archaea bacterial genes, and the first exon of the KIAA0319 gene. Co	390
60	AA480820	Human 1.1 kb mRNA upregulated in retinoic acid treated HL-60 neutrophilic cells	391
61	AA401736	Human ubiquitously-expressed transcript (UXT) mRNA	392
62	N73309	Human signal sequence receptor, γ (translocon-associated protein γ (SSR3), mRNA	393
63	T70352	Homo sapiens mRNA; cDNA DKFZp564O222 (from clone DKFZp564O222)	394
64	AA664020	Homo sapiens mRNA; cDNA DKFZp564M0763 (from clone DKFZp564M0763)	395

65	N55339	Homo sapiens mRNA; cDNA DKFZp564H1916 (from clone DKFZp564H1916)	396
66	N58145	Homo sapiens lipoma HMGIC fusion partner (LHFP) mRNA	397
67	N27165	Homo sapiens clone 24582 mRNA sequence	398
68	T98002	Homo sapiens chromosome 19, cosmid F22329	399
69	AA700688	Homo sapiens ATP synthase, H+ transporting, mitochondrial F1 complex, ε subunit (ATP5E), nuclear gene encoding mitochondrial protein, mRNA	400
70	T41173	Homo sapiens a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1 (ADAMTS1), mRNA	401
71	AA873089	H.sapiens DNA for cyp related pseudogene	402
72	AA487912	guanine nucleotide binding protein (G protein), β polypeptide 1	403
73	AA629909	glycyl-tRNA synthetase	404
74	AA122287	glycoprotein A repetitions predominant	405
75	AA152347	glutathione S-transferase A4	406
76	AA444009	glucosidase, α; acid (Pompe disease, glycogen storage disease type II)	407
77	AA878899	galactosidase, β 1	408
78	N22980	FYN oncogene related to SRC, FGR, YES	409
79	AA865707	fibrinogen, A α polypeptide	410
80	AA679352	farnesyl-diphosphate farnesyltransferase 1	411
81	AA677650	ESTs, Weakly similar to similar to coiled-coil protein [C.elegans]	412
82	AA464143	ESTs, Weakly similar to RNA polymerase II elongation factor ELL2 [H.sapiens]	413
83	AA098892	ESTs, Weakly similar to R12E2.12 [C.elegans]	414
84	N76361	ESTs, Weakly similar to putative Rho/Rac guanine nucleotide exchange factor [H.sapiens]	415
85	AA778640	ESTs, Weakly similar to leucine aminopeptidase [H.sapiens]	416
86	AA488171	ESTs, Weakly similar to formin 4 [M. musculus]	417
87	AA449345	ESTs, Weakly similar to F48E8.2 [C. elegans]	418
88	W73797	ESTs, Weakly similar to Containing ATP/GTP-binding site motif A (P-loop) similar to *C. elegans* protein(P1:CEC47E128); similar to mouse α-mannosidase(P1:B54407) [H.sapiens]	419
89	R67283	ESTs, [H.sapiens]	420
90	AA706829	ESTs, Moderately similar to putative Rab5-interacting protein {clone L1-57} [H.sapiens]	421
91	AA446651	ESTs, Moderately similar to Kryn [M.musculus]	422
92	R16957	ESTs, Highly similar to Jκ recombination signal binding protein [H.sapiens]	423
93	AA149204	ESTs, Highly similar to growth arrest inducible gene product [H.sapiens]	424
94	H73484	ESTs, Highly similar to CGI-106 protein [H.sapiens]	425
95	AA011593	ESTs, Highly similar to cell adhesion regulator [R. norvegicus]	426
96	AA460005	ESTs, Highly similar to antigen NY-CO-33 [H.sapiens]	427
97	AA416627	ESTs	428

98	N80361	ESTs	429
99	AA279648	ESTs	430
100	N49774	ESTs	431
101	R45672	ESTs	432
102	AA463189	ESTs	433
103	AA418040	ESTs	434
104	N34799	ESTs	435
105	AA030013	ESTs	436
106	N36098	ESTs	437
107	N72847	ESTs	438
108	R16545	ESTs	439
109	R91821	ESTs	440
110	AA101971	ESTs	441
111	T52564	ESTs	442
112	AA455962	ESTs	443
113	W69216	ESTs	444
114	N66734	ESTs	445
115	N89738	ESTs	446
116	W84486	ESTs	447
117	AA464603	ESTs	448
118	W31683	ESTs	449
119	AA126673	ESTs	450
120	AA664044	ESTs	451
121	AA149640	ESTs	452
122	AA436184	ESTs	453
123	N24042	ESTs	454
124	AA290631	ESTs	455
125	AA150505	ESTs	456
126	AA009755	ESTs	457
127	AA452118	ESTs	458
128	AA452165	ESTs	459
129	N32226	ESTs	460
130	N92478	ESTs	461
131	N46849	ESTs	462
132	AA460463	ESTs	463

133	T95200	ESTs	464
134	AA454008	ESTs	465
135	H99766	ESTs	466
136	AA191322	ESTs	467
137	AA701521	ESTs	468
138	H41160	ESTs	469
139	AA455094	ESTs	470
140	AA284245	ESTs	471
141	AA197344	ESTs	472
142	R62868	erythrocyte membrane protein band 7.2 (stomatin)	473
143	AA052960	Dyskeratosis congenita 1, dyskerin	474
144	AA621202	DKFZP586D1519 protein	475
145	AA496996	DKFZP564F0522 protein	476
146	AA447502	DKFZP564B147 protein	477
147	AA704226	DKFZP434G162 protein	478
148	R07560	Deoxyguanosine kinase	479
149	AA629999	Cytochrome c oxidase subunit VIIb	480
150	AA486312	Cyclin-dependent kinase 4	481
151	AA292226	Creatine transporter [human, brainstem/spinal cord, mRNA, 2283 nt]	482
152	AA150402	Collagen, type IV, α 1	483
153	AA476282	Coated vesicle membrane protein	484
154	W81562	Cell division cycle 42 (GTP-binding protein, 25kD)	485
155	W81563	Cell division cycle 42 (GTP-binding protein, 25kD)	486
156	AA043228	Calponin 3, acidic	487
157	R76554	Calmodulin 1 (phosphorylase kinase, delta)	488
158	AA487552	Calcium binding atopy-related autoantigen 1	489
159	AA757351	Calcitonin receptor-like	490
160	AA456480	BCL2-like 2	491
161	H14372	ATP-binding cassette, sub-family A (ABC1), member 5	492
162	H38623	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit f, isoform 2	493
163	AA046701	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 1	494
164	AA633658	Amyloid β (A4) precursor protein (protease nexin-II, Alzheimer disease)	495
165	W42849	Amyloid β (A4) precursor protein (protease nexin-II, Alzheimer disease)	496
166	N72918	Adaptor-related protein complex 2, β 1 subunit	497

EXAMPLE IV

Molecular Heterogeneity in CC-RCC

Having identified common alterations in gene expression in CC-RCC tissue, the inventors next sought to identify DNA expression patterns that account for the heterogeneity in the clinical behavior of the disease. Some of the tumors were highly aggressive, leading to patients' deaths within three years, while other patients had no recurrences following surgery (Table 1). The present inventors thus sought to discover gene expression signatures that could identify, predict and possibly account for the lethal tumor phenotype.

A number of methods have been employed to identify DNA expression profiles that were correlated with some observable phenotype or property of cells or tissue. Alizadeh *et al.* (*supra*) performed hierarchical clustering and then searched for selectively expressed groups, while Golub *et al.* (*supra*) and others ranked individual DNAs based on their ability to classify patients (*Science* (2000) **286**:531-7). As discussed in Hastie *et al.*, (GenomeBiology.com (2001) 2, RESEARCH0003), the present strategy called for, first, clustering the DNAs and then assessing the subclusters' ability to differentiate patients. This approach allows exploitation of the value of correlated sets of DNAs and takes advantage of a systematic, mathematical test. The present inventors also performed individual DNA permutation analysis to generate statistical significance values for the ability to make a classification based on an individual DNA.

The inventors compared the expression profiles using a selected set of 3,184 polynucleotides that registered expression ratios greater than 2 (up-or down-regulated) in at least 2 tumors (where results were consistently present in at least 75% of the experiments).

The data was median polished, organized, and visualized using average-linkage hierarchical clustering (Eisen, MB. *et al.*, (1998) *Proc Natl Acad Sci U S A* **95**:14863-4868) (Fig. 6A/Fig. 1). This method arranges DNAs and patients according to similarity in pattern of expression. Many distinct trends in expression were identified by organization of the color patterns in the matrix. However, visual discernment of which clusters are most relevant biologically and clinically was cumbersome.

To circumvent a manual investigation of the correlation between each sub-cluster of DNAs and each clinical parameter, the inventors implemented the program CLUSTERFINDER described above. This program scores and identifies groups of clustered DNAs (nodes in the dendrogram) that best differentiate patients based on a known clinical distinction. The analysis

was biased toward highly correlated DNA clusters by scoring only clusters with >10 DNAs and correlation coefficients >0.5.

The inventors tested two clinical parameters corresponding to two hypotheses of tumor progression. First, "tumor staging" was used as the discriminating clinical parameter, under the assumption that gene expression profiles change as a tumor progresses. The tumors were divided into two groups: (1) stage I and II and (2) stage III and IV. Surprisingly, this distinction did not correlate strongly with any subclusters within the DNA expression matrix.

Second, the inventors used "patient outcome" as the discriminating parameter, under the hypothesis that multiple classes of CC-RCC exist, each having a distinct molecular profile that would correspond to clinical course. For this operation, the inventors distinguished between those patients that died due to cancer within 5 years of initial diagnosis, and those that survived cancer-free for >5 years (Table 1). Also included in the "poor outcome" class were two patients who survived with cancer for 89.4 and 105.6 months. For this "patient outcome" parameter, multiple clusters of DNAs distinguished classes of patients. Cluster 687, containing 24 DNAs, and its parent, Cluster 1281, containing 51 DNAs, had the highest predictive scores (1.70). Cluster 3014, with 48 DNAs, and cluster 2199, with 61 DNAs, also had strong predictive scores (1.46, 1.011).

Figs 6B, 6C and Figures 2A, 2B, 3A, 3B, 4A and 4B depict the re-clustering of patients based on these subclusters. Cluster 1281 displays marked separation of the two classes of patients, with the exception of patient 30. Cluster 3014 also separates the patients well, although expression values within this cluster did not correlate as highly.

The significance of this underlying molecular profile was confirmed using a modified permutation t-test. 217 DNAs differentiated the two outcome groups significantly ($\alpha<0.001$). All 51 DNAs within Cluster 1281 (see also Table 6) were present in this group of 217 DNAs (Table 6 + Table 7).

Thus, Table 6 shows the 51 sequences of greatest interest in their ability to distinguish between the two clinical types of CC-RCC discerned by the present inventors: aggressive and non-aggressive. Table 6A shows 28 genes (SEQ ID NO:1-21 and SEQ ID NO:139) whose expression is upregulated non-aggressive cases of CC-RCC (tumor compared to normal tissue). In contrast, Table 6B lists 23 genes (apparently 19 unique sequences designated SEQ ID NO:22-39) that are down-regulated in aggressive CC-RCC (tumor tissue relative to normal kidney tissue). On the basis of these expression patterns of as few as 1 gene and as many as all 51 gene probes (apparently 39 or 40 unique sequences; SEQ ID NO:1-39 and SEQ ID NO:139), it is

possible to obtain a molecular classification of CC-RCC into the two clinically distinct classes. This serves as the basis of an routine molecular prognostic assay that can be done to classify CC-RCC patients and tailor their therapy and follow-up programs in accordance with their prognosis.

EXAMPLE V

Clinical Simulation

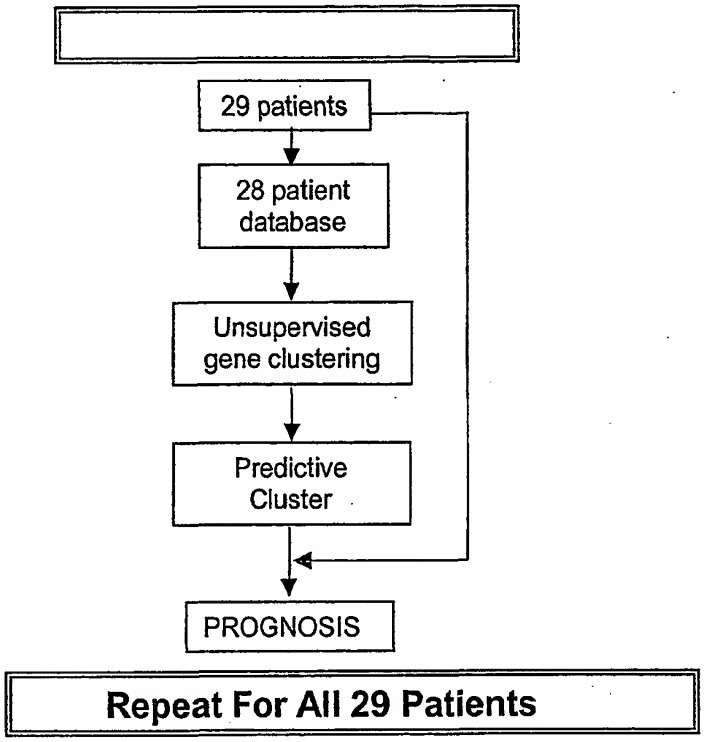
These discriminating clusters of DNAs have at least two applications: providing insight into potential molecular subtypes of CC-RCC, and as a means for objective and accurate determination of patient prognosis. To address the second, the present inventors performed a clinical simulation. Because the DNAs in these identified clusters were ordered using the molecular profiles of the 29 patients, testing the predictive ability of these DNAs on the same 29 patients would be biased. To remove this bias, each patient's data was systematically treated as if it came from an unknown test patient who had just undergone nephrectomy and a molecular profile screening with the present cDNA probe set, while the remaining 28 patients served to populate the database of known molecular profiles/clinical follow-up data. The same analysis protocol described above was followed independently of the test patient.

A flow diagram of the simulation process is shown below.

By sequential removal of any individual patient (being treated as an "unknown") from the clusters, the clustering of DNAs was slightly altered so that the clusters were no longer identical in structure to the originally predictive clusters. Throughout this simulation, the set of DNAs identified as Cluster 1281 consistently clustered together, as expected from their high correlation index in the original clustering operation. Although a few DNAs appeared in this grouping sporadically, on average, 95% of the DNAs in the original cluster were also present in the simulation clusters identified by CLUSTERFINDER. However, DNAs in the other previously identified clusters did not maintain their order during the simulation. This follows from the fact that these other clusters did not have as high correlation indices in the original operation.

Since the clusters containing DNAs similar to Cluster 1281 maintained high predictive scores and consistent DNA content throughout the simulation, the inventors used these as predictive tests for the respective "test" patients. The test patient's prognosis was predicted by

comparing his profile with that of the independently established predictive cluster (*i.e.*, from which the "test" patient's data had been removed).



Remarkably, the clusters of DNAs similar to those in Cluster 1281 independently permitted correct prediction of patient outcomes correctly in all but one case. This one prediction failure was a patient with advanced stage cancer who survived >5 years. The test never failed to predict patients with poor outcomes. The comparison of patient prognosis based on staging vs. molecular profiling is presented in the two rightmost columns of Table 1.

EXAMPLE VI

Content of DNAs in the Predictive Cluster

Since the DNAs within Cluster 1281 proved predictive and stable throughout the simulation, the inventors investigated the DNAs within this cluster and their potential implication in the biology of the highly aggressive sub-type of CC-RCC.

Tables 2 and 3 present a subset of 123 genes that are generally up-regulated in CC-RCC tissue versus normal kidney tissue. Table 2 shows the most consistently and/or strongly

upregulated "first" (most preferred) subset of genes (SEQ ID NO: 40-68). These genes are up-regulated at least 3-fold in 75% or more of the CC-RCC patients. Table 3 shows a second set of 91 up-regulated genes (SEQ ID NO:140-230) which are up-regulated at least 2-fold in 75% or more of the CC-RCC patients.

Tables 4 and 5 present a subset of 178 genes that are down-regulated in CC-RCC tissue versus normal kidney tissue. Table 4 shows the 77 most consistently and/or strongly down-regulated "first" (most preferred) subset of genes (SEQ ID NO:69-138). These genes are down-regulated by at least 3-fold in 75% or more of the CC-RCC patients. Table 5 shows a second set of 101 down-regulated genes (SEQ ID NO:231-331) that are down-regulated by at least 2-fold in 75% or more of the CC-RCC patients.

The gene products (taken from serum, urine, saliva, or other abundant body fluid rather than kidney tissue) of the up-regulated expressed nucleic acids (Tables 2 and 3) can be assayed using in immunoassays known in the art (*i.e.*, ELISA, immunocytochemistry, sandwich assays, *etc.*) for the purpose of diagnosing patients with CC-RCC but do not discriminating between the heterogeneous disease severity.

Differentially expressed nucleic acids indicative of aggressive versus non-aggressive disease phenotype are not included in this subset but were independently determined by the inventors through clustering and t-statistics. The list of expressed nucleic acids discovered to be indicative are summarized in Fig. 6A and Tables 6 and 7. Figure 5 represents the actual relative expression values for the 51 cDNAs that comprise Cluster 1281. (See also Table 6.) The inventors have shown that these 51cDNAs are down regulated in the aggressive CC-RCC phenotype (SEQ ID NO:22-39) or up-regulated in non-aggressive CC-RCC (SEQ ID NO:1-21 and 139). A larger set of genes that are differentially expressed in aggressive vs. non-aggressive CC-RCC includes the 166 probes SEQ ID NO:332-497, inclusive. It is not yet clear how these genes break down into the two categories so far identified in these prognostic genes.

These two classes of gene can be viewed as

(A) positive effectors of less aggressive CC-RCC; and

(B) inhibitors of tumor progression that would keep less aggressive CC-RCC in check.

For example PDGFR expression was said to be an indicator of proliferation in other cancers (Lafuente, *et al.*. (1999) *J Mol Neurosci* 13:177-85) while *sprouty* homologue 1 (*D. melanogaster*) negatively modulates angiogenesis by inhibiting tyrosine kinase-mediated

signaling pathways (Lee, SH *et al.* (2000) *J Biol Chem* 26:26) such as the VEGF pathway. It is noteworthy that VEGF was highly up regulated in all CC-RCC cases tested.

The DNAs in Group II are almost exclusively down regulated in the highly aggressive CC-RCC cases. This group includes TGFβRII, TIMP3, and insulin-like growth factor binding protein 7 (IGF-BP7). All of these genes/proteins have been implicated in late-stage or aggressive cancer.

EXAMPLE VII

Expression of Specific Genes in CC-RCC

Ceruloplasmin, a protein involved in iron and copper homeostasis, had the highest increase in expression in CC-RCC vs. normal tissue. Interestingly, only a handful of reports showed an association between ceruloplasmin and CC-RCC. One study reported secretion of this protein by CC-RCC (Saito, K., *et al.* (1985) *Biochem Med* 33:45-52) and the other reported its elevation in RCC patient serum (Pejovic, M. *et al.* (1997) *Int Urol Nephrol* 29:427-32). The present discovery merits an in-depth investigation of ceruloplasmin's role in CC-RCC tumorigenesis and its potential value as a tumor marker.

Another copper-related protein, lysyl oxidase (11-fold up-regulated in 95% of CC-RCC) is an extracellular enzyme involved in connective tissue maturation. It is highly expressed in invasive breast cancer cell lines (Kirschmann, DA *et al.*, (1999) *Breast Cancer Res Treat* 55:127-136) but has never been studied in RCC.

Finally, a well-known angiogenic factor, VEGF, has been shown to be highly expressed in RCC (Takahashi, A *et al.*, (1994) *Cancer Res* 54:4233-4237; Thelen, P *et al.*, (1999) *Anticancer Res* 19:1563-1565) and elevated in the serum of RCC patients (Sato, K *et al.*, (1999) *Jpn J Cancer Res* 90:874-879; Wechsel, HW *et al.*, (1999) *Anticancer Res* 19:1537-540). The present invention corroborated those observations and showed an average 5-fold up-regulation of VEGF in 96% of the CC-RCC tumors.

The present invention also identified a large number of examples of prominent down-regulation of DNAs in CC-RCC. Most strikingly, kininogen was more than 27-fold down-regulated. This protein, involved in the activation of the clotting system, has recently been shown to be anti-angiogenic (Zhang, JC *et al.* (2000) *FASEB J* 14: 2589-600). Its down regulation, never before reported in CC-RCC, in combination with the up-regulation of VEGF might explain the characteristic hypervascularization of CC-RCC.

The metallothionein (MT) family of genes was coordinately down regulated in CC-RCC. The products of these genes modulate the release of hydroxyl radicals and the exchange of heavy metals such as zinc, cadmium or copper. Differential expression of this class of genes has been reported in many cancers (Janssen, *et al.*, (2000) *J Pathol* **192**:293-300). Several subtypes, MT-1A, MT-1G, MT-1H were reported to be down regulated in RCC (Nguyen *et al.*, (2000) *Cancer Lett* **160**:133-40; Izawa, *et al.*, (1998) *Urology* **52**:767-72). The present invention supports these reports and adds the fact that MT-1L and MT-1E were down-regulated.

Based on this model, the present inventors conceived that a distinctive molecular profile exists early in tumor development. The more aggressive type tumor progresses much more rapidly, and thus usually presents clinically at a more advanced stage, while tumors of the less aggressive class progress slowly and usually present clinically before tumor cells have invaded sites outside the kidney. This model is strongly supported by the dataset disclosed herein. Indeed, only one patient with CC-RCC having the aggressive molecular signature survived >5 years. This patient presented with stage III cancer, but 7 years later, had no evidence of disease ("NED"; Patient 30, Table 1).

Remarkably, the "molecular signature" approach of the present invention was of sufficient robustness to predict correctly the outcome in five cases in which the clinico-pathological information would have suggested otherwise.

One patient with the non-aggressive molecular signature had, at surgery, a grade 3 tumor invading the renal vein, but has since survived for 7.5 years (Patient 29, Table 1).

Another patient, with a stage II, grade 2 tumor went on to die of the cancer 4.6 years after surgery (Patient 55, Table 1 and Figures). Using the present molecular signature, the latter patient was classified as having the aggressive subtype.

Another patient with bone metastasis at diagnosis is still alive after 8.8 years and survives despite the bone metastasis, which is stable (Patient 54, Table 1 and Figures). Using the present approach, this patient was identified as having the non-aggressive molecular signature.

These cases and others demonstrate that the set of genes described herein, or a subset thereof, is useful in determining the prognosis of patients with CC-RCC.

Loss of the TGFβII signaling pathway in late stages of RCC has previously been shown.

TIMP3 is known to be downstream of TGFβ and is a known tumor suppressor gene. By inhibiting the function of matrix metalloproteinases, TIMP3 regulates cell adhesion and

extracellular matrix homeostasis. Loss of TIMP3 expression by promoter methylation was shown to increase tumorigenicity due to unregulated MMPs (Bachman, *et al.*, (1999) *Cancer Res* **59**:798-802).

The present out clustering methodology has effectively demonstrated correlation of an entire pathway and its exclusive down regulation in the aggressive cancers. The ligands, the receptors and a downstream effectors are all down regulated and all are implicated in aggressive cancer.

The references cited above are all incorporated by reference herein, whether specifically incorporated or not.

CLAIMS

1. A microarray useful as a prognostic composition, comprising a matrix of at least one cDNA probe from a set of probes immobilized to a solid surface in predetermined order such that a row of pixels corresponds to replicates of one distinct probe from the set, the probes being any of SEQ ID NO:1 — SEQ ID NO:39 inclusive, SEQ ID NO:139 or SEQ ID NO:332 — SEQ ID NO:497, inclusive; and

wherein the probes are complementary to nucleic acid sequences expressed differentially in aggressive as compared to non-aggressive types of clear cell renal cell carcinoma (CC-RCC), which nucleic acid sequences hybridize to the probes under high stringency conditions.

2. The microarray of claim 1, wherein the set of probes comprises at least 10 cDNA probes, which probes have the sequence SEQ ID NO:1—SEQ ID NO:10.

3. The microarray of claim 1, wherein the set of probes comprises at least 39 cDNA probes, which probes have the sequence SEQ ID NO:1 — SEQ ID NO:39.

4. The microarray of claim 1, wherein the set of probes comprises at least 206 cDNA probes, which probes have the sequence SEQ ID NO:1 — SEQ ID NO:39, SEQ ID NO:139 and SEQ ID NO:332 — SEQ ID NO:497.

5. The microarray of any of claims 1-4, wherein the one or more probes comprise nucleotides having at least one modified phosphate backbone selected from a phosphorothioate, a phosphoridothioate, a phosphoramidothioate, a phosphoramidate, a phosphordiimidate, a methylsphosphonate, an alkyl phosphotriester, 3'-aminopropyl, a formacetal, or an analogue thereof.

6. The microarray of claim 1 or 5, wherein each probes comprises at least 15 nucleotides.

7. The microarray of any of claims 1-6, further comprising one or more nucleic acid samples representing expressed genes, each sample from an individual subject's tumor or normal tissue, each sample spotted column-wise on the pixels of the microarray probes.

8. The microarray of claim 7, which has further been subjected to nucleic acid hybridization under high stringency conditions such that the nucleic acid samples are hybridized with the immobilized probes on which the samples have been spotted.

9. A composition comprising a set of two or more oligonucleotide or polynucleotide probes each of which hybridizes with part or all of a coding sequence that is differentially expressed in CC-RCC tumors compared to normal kidney tissue.

10. The composition of claim 9 comprising a set of at least 10 of the probes.

11. The composition of claim 10 comprising a set of at least 99 of the probes.

12 The composition of claim 11 comprising a set of at least 291 of the probes.

13. A composition comprising a set of two or more oligonucleotide or polynucleotide probes each of which hybridizes with part or all of a coding sequence that is differentially expressed in an aggressive type of CC-RCC compared to a non-aggressive type of CC-RCC.

14. The composition of claim 13 comprising a set of at least 10 of the probes.

15. The composition of claim 14 comprising a set of at least 39 of the probes.

16. The composition of claim 15 comprising a set of at least 206 of the probes.

17. The composition of any of claims 13-16, wherein the coding sequence is up-regulated in the aggressive CC-RCC compared to normal kidney tissue.

18. The composition of any of claims 13-16, wherein the coding sequence is down-regulated in the aggressive CC-RCC compared to normal kidney tissue.

19. The composition of any of claims 13-16, wherein the coding sequence is up-regulated in the non-aggressive CC-RCC compared to normal kidney tissue.

20. The composition of any of claims 13-16, wherein the coding sequence is down-regulated in the non-aggressive CC-RCC compared to normal kidney tissue.

21. The composition of any of claims 9-16, wherein the probes are of mammalian origin.

22. The composition of claim 21 wherein the probes are of human origin.

23. A method of predicting whether a subject with a CC-RCC has non-aggressive or aggressive-type CC-RCC, comprising the steps of:

(a) examining the expression in tumor tissue from the subject of nucleic acid that hybridizes at high stringency conditions with one or more oligonucleotide or

polynucleotide probes having the sequence of SEQ ID NO:1 through SEQ ID NO:21, inclusive;

(b) examining the expression in normal kidney tissue of the subject of nucleic acid that hybridizes at high stringency conditions with the oligonucleotide or polynucleotide probes of step (a);

(c) comparing the expression in tumor tissue in step (a) with the expression in normal tissue in step (b),

wherein, when the expression is up-regulated at least 2-fold in the tumor tissue compared to the normal kidney tissue, the CC-RCC is non-aggressive.

24. The method of claim 23, wherein the nucleic acid from the tumor or normal tissue is labeled with a detectable label.

25. The composition of claim 24, wherein the detectable label is a fluorescent label.

26. The method of any of claims 23-25, wherein, when the expression is up-regulated at least 3-fold in the tumor tissue compared to the normal kidney tissue, the CC-RCC is non-aggressive.

27. The method of any of claims 23-25, wherein, when the expression is up-regulated at least 4-fold in the tumor tissue compared to the normal kidney tissue, the CC-RCC is non-aggressive.

28. A method of predicting whether a subject with CC-RCC has non-aggressive or aggressive-type CC-RCC, comprising the steps of:

(a) examining the expression in tumor tissue from the subject of nucleic acid that hybridizes at high stringency conditions with one or more oligonucleotide or polynucleotide probes having the sequence of SEQ ID NO: 22 through SEQ ID NO:39 inclusive;

(b) examining the expression in normal kidney tissue of the subject of nucleic acid that hybridizes at high stringency conditions with the oligonucleotide or polynucleotide probe of step (a);

(c) comparing the expression in tumor tissue in step (a) with the expression in normal tissue in step (b),

wherein, when the expression is down-regulated at least 2-fold, in the tumor tissue compared to the normal kidney tissue, the CC-RCC is aggressive.

29. The method of claim 28, wherein, when the expression is down-regulated by at least 3-fold, in the tumor tissue compared to the normal kidney tissue, the CC-RCC is aggressive.

30. The method of claim 27, wherein, when the expression is down-regulated by at least 4-fold, in the tumor tissue compared to the normal kidney tissue, the CC-RCC is aggressive.

31. The method of any of claims 23-30 wherein

(a) the nucleic acid from the tumor and the tissue is labeled with a fluorescent label prior to the hybridization; and

(b) the hybridization is detected as a fluorescent signal bound to the probe.

32. The method of any of claims 23 - 31 wherein

(a) the probes are immobilized to a solid surface in a microarray as pixels arranged in rows; and

(b) the tumor tissue or normal kidney tissue samples are spotted column-wise onto the probe pixels.

33. A method for early diagnosis of a CC-RCC tumor in a subject prior to physical or radiological evidence of the tumor, comprising the steps of:

(a) selecting a protein product of at least one gene the expression of which is up-regulated in a majority of CC-RCC patients, which protein is a secreted protein or is expressed on cell surfaces in a tissue that is readily accessible for assay; and

(b) determining the presence or measuring the quantity of the protein product in a body fluid or a tissue or cell sample from the subject,

wherein, an increased level of the protein product compared to

(i) the level in a normal subject's fluid, tissue or cells, or

(ii) another reference normal value is indicative of the presence of a CC-RCC tumor in the subject.

34. A method for diagnosing the recurrence of a CC-RCC tumor in a subject in whom a CC-RCC primary tumor has been excised or otherwise treated, comprising the steps of:

(a) selecting a protein product of at least one gene the expression of which is up-regulated in a majority of CC-RCC patients, which protein is a secreted protein or is expressed on cell surfaces in a tissue that is readily accessible for assay; and

(b) determining the presence or measuring the quantity of the protein product in a body fluid or a tissue or cell sample from the subject,

wherein, an increased level of the protein product compared to

(i) the level in a normal subject's fluid, tissue or cells, or

(ii) another reference normal value,

is indicative of the presence of a recurrent CC-RCC tumor in the subject.

35. The method of claim 33 or 34 wherein the gene is one that hybridizes with any one or more of SEQ ID NO:40 — SEQ ID NO:68 or SEQ ID NO:140 — SEQ ID NO:230.

36. The method of claim 35 wherein the gene is one that hybridizes with one or more of SEQ ID NO:40 — SEQ ID NO:68

37. A kit comprising:

(a) the microarray of any of claims 1-8;

(b) reagents that facilitate hybridization of the nucleic acid to the immobilized probes; and

(c) a computer readable storage medium comprising logic which enables a processor to read data representing detection of hybridization.

38. A kit comprising:

(a) the composition of any of claims 9-22;

(b) reagents that facilitate hybridization of the nucleic acid to the immobilized probes; and

(c) a computer readable storage medium comprising logic which enables a processor to read data representing detection of hybridization.

39. The kit of claims 37 or 38 wherein the reagents are ones that facilitate detection of fluorescence.

40. A kit comprising:

(a) the microarray or composition of any of claims 1-22;

(b) means for carrying out hybridization of the nucleic acid to the probes; and

(c) means for reading hybridization data.

41. The kit of claim 40, wherein the hybridization data is in the form of fluorescence data.

42. The kit of claims 40 or 41 wherein the probes are immobilized to the microarray.

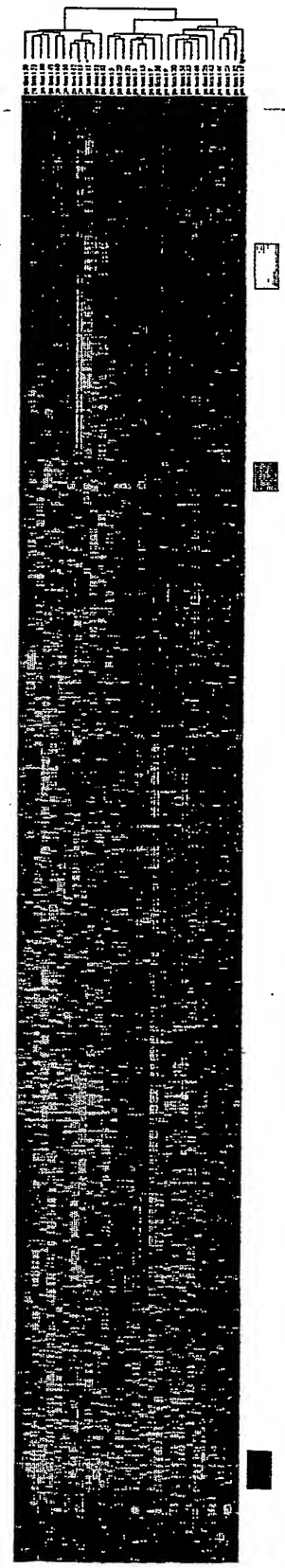
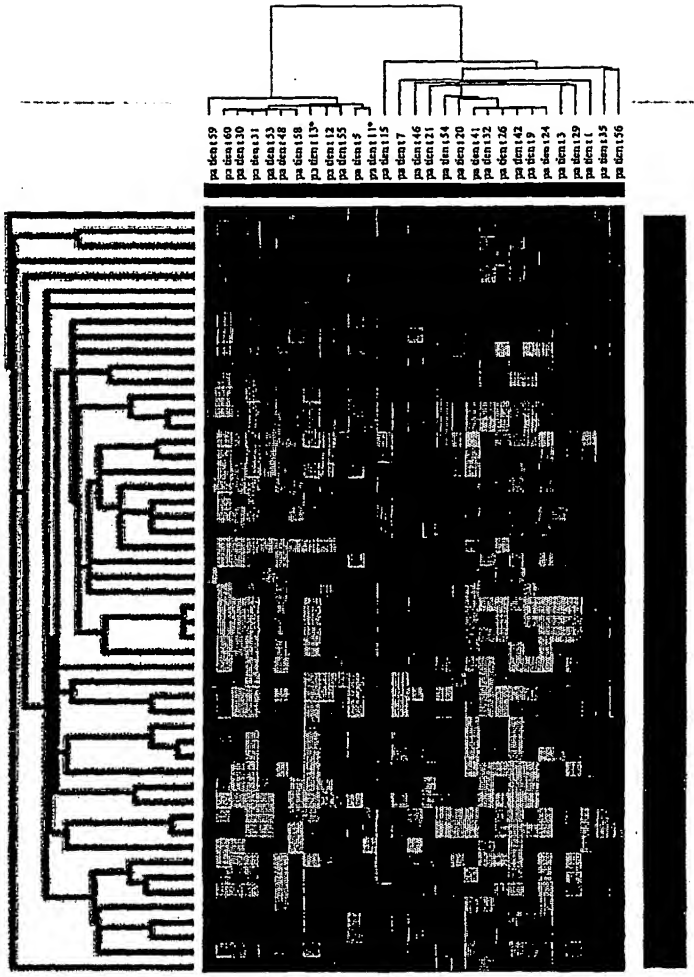


Fig. 1



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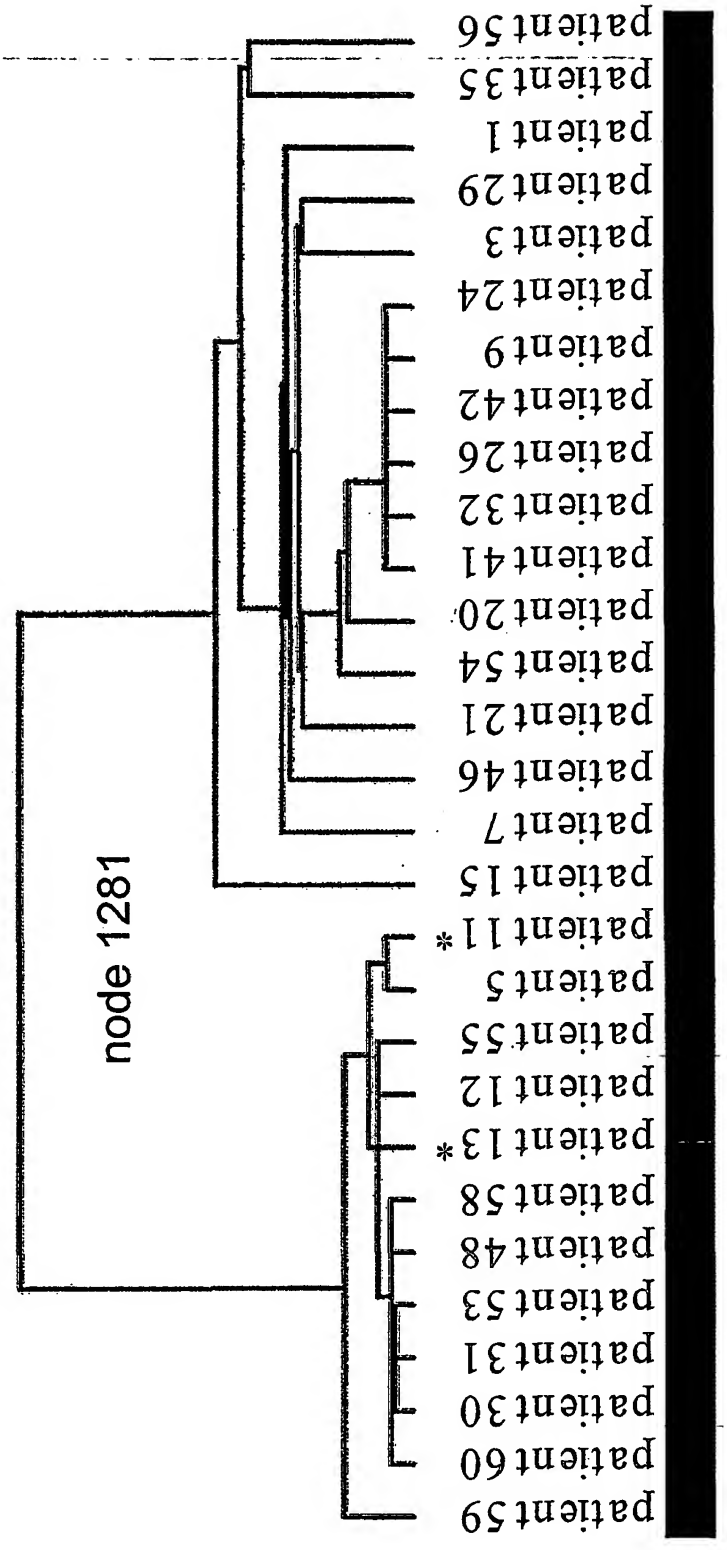
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Fig. 2B

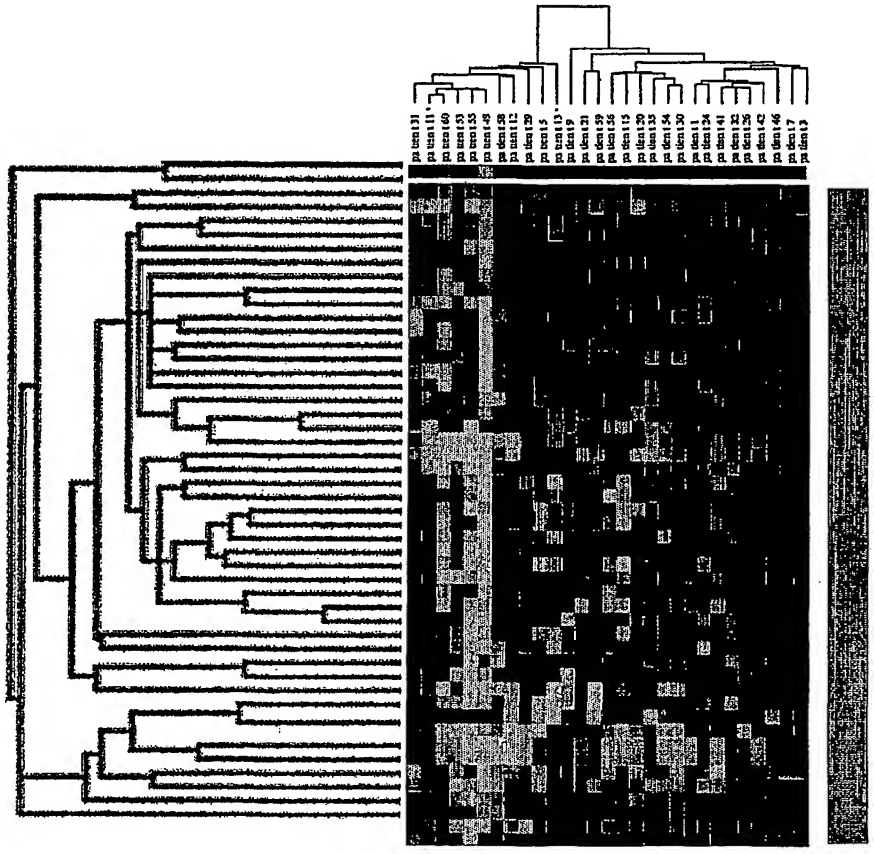

Fig. 3A


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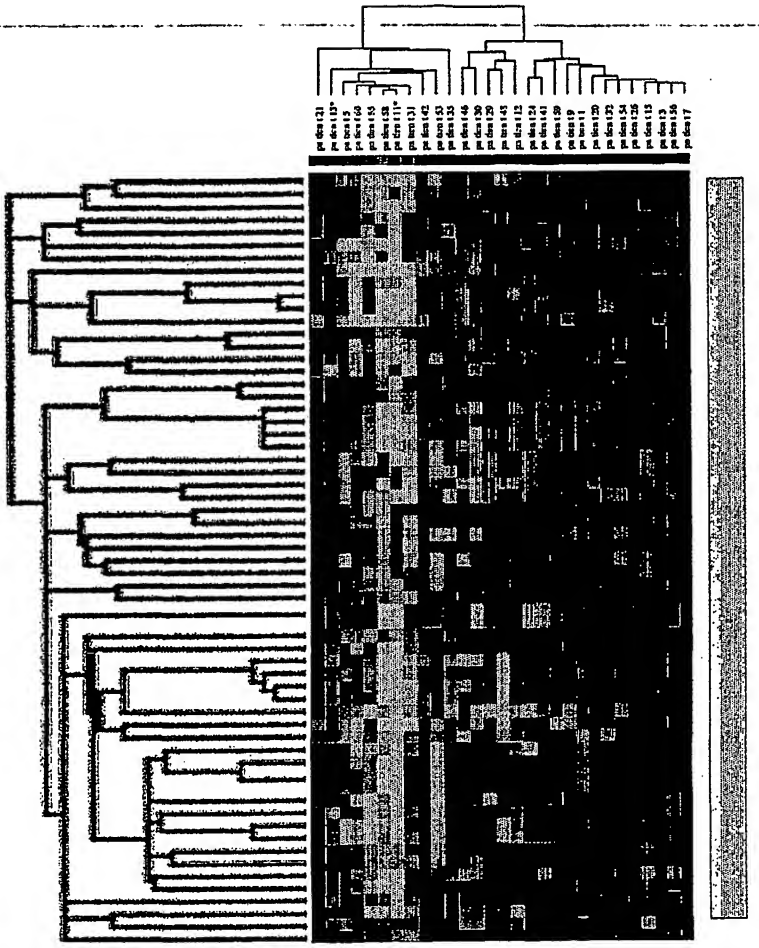
Fig. 3B



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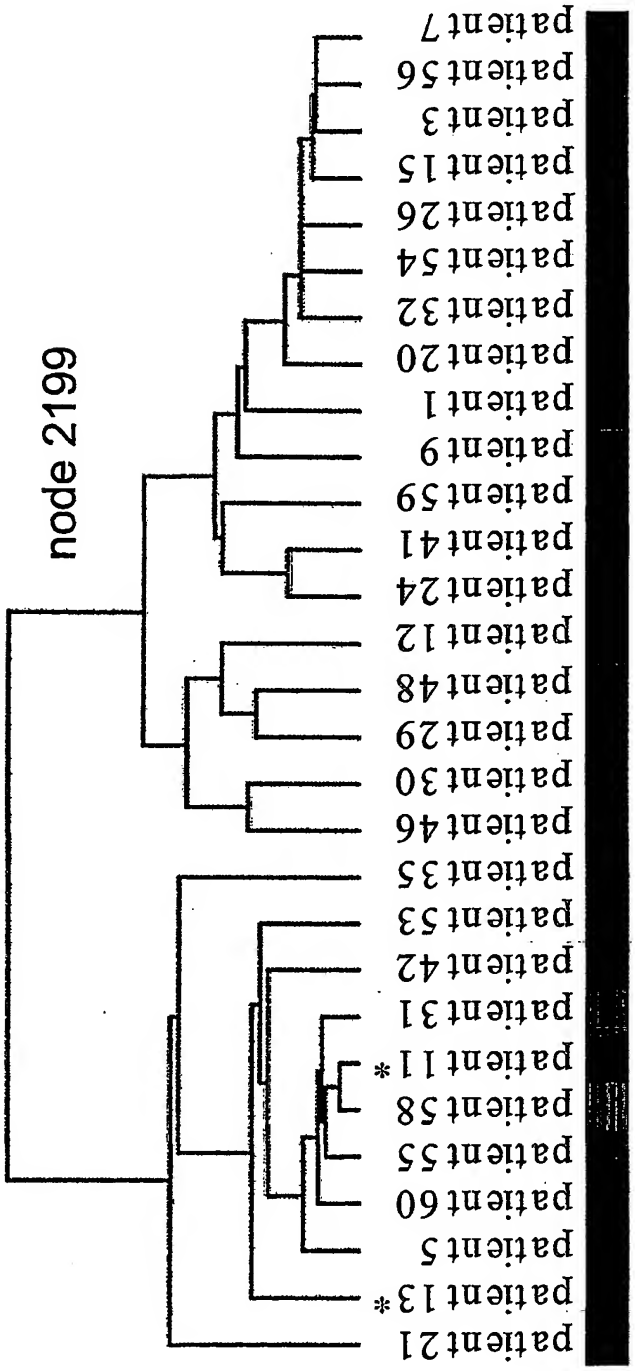
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Fig. 4B

Fig. 5

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AA001614	insulin receptor
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AA490694	hevin
AA159194	FAT tumor suppressor (Drosophila) homolog
AA485082	serum/glucocorticoid regulated kinase
W60845	cell division cycle 42 (GTP-binding protein, 25kD)
N34362	regulator of G-protein signalling 5
AA568470	regulator of G-protein signalling 5
R54815	Rab9 effector p40
AA469136	transcription factor 4
W72803	ESTs, Weakly similar to KIAA0768 protein [H.sapiens]
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H72113	CD34 antigen
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AA055440	sprouty (Drosophila) homolog 1 (antagonist of FGF signaling)
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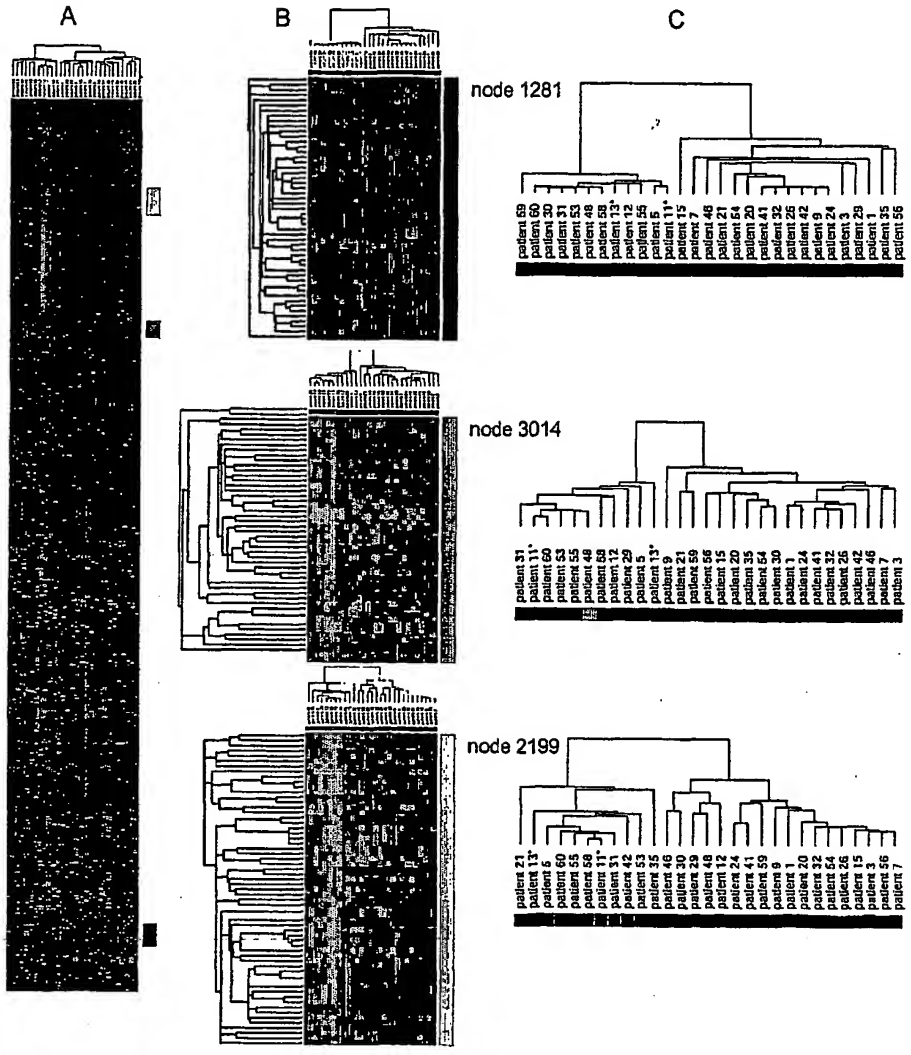
Fig. 6



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<120> Microarray Gene Expression Profiling in Clear Cell Renal Cell Carcinoma:
Prognosis and Drug Target Identification

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<213> Homo sapiens

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<212> DNA
<213> Homo sapiens

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<211> 2193
<212> DNA
<213> Homo sapiens

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<210> 36
<211> 354
<212> DNA
<213> Homo sapiens

<400> 36
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<211> 2272
<212> DNA
<213> Homo sapiens

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<211> 594
<212> DNA
<213> Homo sapiens

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<210> 39
<211> 942
<212> DNA
<213> Homo sapiens

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<210> 40
<211> 3323
<212> DNA
<213> Homo sapiens

<400> 40
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<211> 927

<212> DNA
<213> Homo sapiens

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<212> DNA
<213> Homo sapiens

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<211> 1821
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<213> Homo sapiens

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<211> 996
<212> DNA
<213> Homo sapiens

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<210> 47
<211> 781
<212> DNA
<213> Homo sapiens

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<211> 876
<212> DNA
<213> Homo sapiens

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<211> 701
<212> DNA
<213> Homo sapiens

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<210> 50
<211> 909
<212> DNA
<213> Homo sapiens

<400> 50
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<212> DNA
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<213> Homo sapiens

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<213> Homo sapiens

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<213> Homo sapiens

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<213> Homo sapiens

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<212> DNA
<213> Homo sapiens

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<212> DNA

<213> Homo sapiens

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<212> DNA
<213> Homo sapiens

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<211> 787
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<213> Homo sapiens

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<213> Homo sapiens

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<400> 139

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